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<b>(21) International Application Number:</b> PCT/IB99/00103  <b>(22) International Filing Date:</b> 14 January 1999 (14.01.99)  <b>(30) Priority Data:</b> 9800760.2                      14 January 1998 (14.01.98)                      GB 9819015.0                      1 September 1998 (01.09.98)                      GB 9822143.5                      9 October 1998 (09.10.98)                      GB  <b>(71) Applicant (for all designated States except US):</b> CHIRON S.P.A. [IT/IT]; Via Fiorentina, 1, I-53100 Siena (IT).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MASIGNANI, Vega [IT/IT]; Via Pantaneto, 105, I-53100 Siena (IT). RAP- PUOLI, Rino [IT/IT]; Via delle Rocche, 1, Vagliagli, I-53019 Castelnuovo Berardenga (IT). PIZZA, Mariagrazia [IT/IT]; Strada di Montalbuccio, 160, I-53100 Siena (IT). SCARLATO, Vincenzo [IT/IT]; Via Firenze, 3/37, I-53134 Colle Val d'Elsa (IT). GRANDI, Guido [IT/IT]; 9° Strada, 4, I-20090 Segrate (IT).  <b>(74) Agent:</b> HALLYBONE, Huw, George; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished          upon receipt of that report.</i>
<b>(54) Title:</b> NEISSERIA MENINGITIDIS ANTIGENS		
<b>(57) Abstract</b>  The invention provides proteins from <i>Neisseria meningitidis</i> (strains A and B), including amino acid sequences, the corresponding nucleotide sequences, expression data, and serological data. The proteins are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.		

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## NEISSERIA MENINGITIDIS ANTIGENS

This invention relates to antigens from the bacterium *Neisseria meningitidis*.

**BACKGROUND**

*Neisseria meningitidis* is a non-motile, gram negative diplococcus human pathogen. It colonises the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N.gonorrhoeae*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

*N.meningitidis* causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants [*eg.* Morbidity and Mortality weekly report, Vol.46, No. RR-5 (1997)]. This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the

vaccination against *H.influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease" in: *New Generation Vaccines, supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A and C. *Vaccine* 10:691-698).

Meningococcus B remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of  $\alpha(2-8)$ -linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to menB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (eg. Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (eg. Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (eg. EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that

are presumed targets for the immune system and which are not antigenically variable. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*.

## 5 THE INVENTION

The invention provides proteins comprising the *N.meningitidis* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences homologous (*ie.* having sequence identity) to the *N.meningitidis* amino acid sequences disclosed in the examples. Depending on the particular  
10 sequence, the degree of sequence identity is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more). These homologous proteins include mutants and allelic variants of the sequences disclosed in the examples. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between the proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH  
15 program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

The invention further provides proteins comprising fragments of the *N.meningitidis* amino acid sequences disclosed in the examples. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (*eg.* 8, 10, 12,  
20 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (*eg.* recombinant expression, purification from cell culture, chemical synthesis *etc.*) and in various forms (*eg.* native, fusions *etc.*). They are preferably prepared in substantially pure form (*ie.* substantially free from other *N.meningitidis* or host cell proteins)

25 According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means.

According to a further aspect, the invention provides nucleic acid comprising the *N.meningitidis* nucleotide sequences disclosed in the examples. In addition, the invention provides nucleic acid comprising sequences homologous (*ie.* having sequence identity) to the *N.meningitidis* nucleotide sequences disclosed in the examples.

- 5 Furthermore, the invention provides nucleic acid which can hybridise to the *N.meningitidis* nucleic acid disclosed in the examples, preferably under “high stringency” conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least *n* consecutive nucleotides from the *N.meningitidis* sequences and, depending on the  
10 particular sequence, *n* is 10 or more (*eg.* 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (*eg.* for antisense or probing purposes).

- 15 Nucleic acid according to the invention can, of course, be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*).

In addition, the term “nucleic acid” includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

- 20 According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*eg.* expression vectors) and host cells transformed with such vectors.

According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (eg. as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria. Said Neisserial bacteria may be any species or strain (such as *N.gonorrhoeae*) but are preferably *N.meningitidis*, especially strain A, strain B or strain C.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Unlike the sequences disclosed in PCT/IB98/01665, the sequences disclosed in the present application are believed not to have any significant homologs in *N.gonorrhoeae*. Accordingly, the sequences of the present invention also find use in the preparation of reagents for distinguishing between *N.meningitidis* and *N.gonorrhoeae*

A summary of standard techniques and procedures which may be employed in order to perform the invention (eg. to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

5 General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature eg. Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and*  
10 *ii* (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene*  
15 *Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

20 Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference. In particular, the contents of UK patent applications 9800760.2, 9819015.0 and 9822143.5 are incorporated herein.

Definitions

25 A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.



The term “comprising” means “including” as well as “consisting” *eg.* a composition “comprising” X may consist exclusively of X or may include something additional to X, such as X+Y.

The term “heterologous” refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the  
5 heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

10 An “origin of replication” is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain  
15 origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A “mutant” sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the  
20 degree of sequence identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an “allelic variant” of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second  
25 isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5’ or 3’ untranslated regions of the gene, such as in regulatory control regions (*eg.* see US patent 5,753,235).

### Expression systems

The Neisserial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

#### i. Mammalian Systems

- 5 Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription
- 10 initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A*
- 15 *Laboratory Manual, 2nd ed.*].

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-

20 viral genes, such as the murine metallothionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive cells.

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can

25 stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science* 236:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements

30 derived from viruses may be particularly useful, because they usually have a broader host range.

Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J.* 4:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) *Proc. Natl. Acad. Sci.* 79:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell* 41:521]. Additionally, some enhancers are regulatable and become active only  
5 in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet.* 2:215; Maniatis et al. (1987) *Science* 236:1237].

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired,  
10 the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo*  
15 or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells  
20 are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell* 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem.*  
25 *Sci.* 14:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as  
5 an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell* 23:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T  
10 antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol.* 9:946] and pHEBO [Shimizu et al.  
15 (1986) *Mol. Cell. Biol.* 6:1074].

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct  
20 microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*eg.* Hep G2), and a  
25 number of other cell lines.

## ii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include  
30 a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus

genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

- 5 After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit).
- 10 These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

- Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and
- 15 transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable
- 20 maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

- Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and
- 25 which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human  $\alpha$ -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacqz-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays* 4:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15  $\mu\text{m}$  in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of recombinant

virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*,  
5 *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol.* 25:225).

Cells and cell culture media are commercially available for both direct and fusion expression of  
10 heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See, eg.* Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced.  
15 Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, *eg.* HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the  
20 product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, *eg.* proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence.  
25 These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

### iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as:



US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., *Nucleic Acids Research* 15:2515-2535 (1987); Wirsal et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, *Gibberellins*: in: *Advanced Plant Physiology*, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for *Agrobacterium* transformations, T DNA sequences for *Agrobacterium*-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Repr.*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward

antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet.*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high

velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the

history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

#### iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in *Escherichia coli* (*E. coli*) [Raibaud *et al.* (1984) *Annu. Rev. Genet.* 18:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature* 198:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.*

(1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature* 292:128] and T5 [US patent 4,689,406]  
5 promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac*  
10 promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene* 25:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci.* 80:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase  
15 to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol.* 189:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci.* 82:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

20 In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature* 254:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the  
25 pairing of bases between the SD sequence and the 3' end of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature* 309:810]. Fusion proteins can also be made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene* 60:197], *trpE* [Allen *et al.* (1987) *J. Biotechnol.* 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol.* 135:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology* 7:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghayeb *et al.* (1984) *EMBO J.* 3:2437] and the *E. coli* alkaline

phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci.* 82:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 244 042].

- 5 Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription.
- 10 Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

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Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

25

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol.* 32:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: *Bacillus subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], *Escherichia coli* [Shimatake *et al.* (1981) *Nature* 292:128; Amann *et al.* (1985) *Gene* 40:183; Studier *et al.* (1986) *J. Mol. Biol.* 189:113; EP-A-0 036 776, EP-A-0 136 829 and EP-A-0 136 907], *Streptococcus cremoris* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655]; *Streptococcus lividans* [Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655], *Streptomyces lividans* [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with  $\text{CaCl}_2$  or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *eg.* [Masson *et al.* (1989) *FEMS Microbiol. Lett.* 60:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, *Bacillus*], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci.* 85:856; Wang *et al.* (1990) *J. Bacteriol.* 172:949, *Campylobacter*], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci.* 69:2110; Dower *et al.* (1988) *Nucleic Acids Res.* 16:6127; Kushner (1978) "An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol.* 53:159; Taketo (1988) *Biochim. Biophys. Acta* 949:318; *Escherichia*], [Chassy *et al.* (1987) *FEMS Microbiol. Lett.*



44:173 *Lactobacillus*]; [Fiedler *et al.* (1988) *Anal. Biochem* 170:38, *Pseudomonas*]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett.* 66:203, *Staphylococcus*], [Barany *et al.* (1980) *J. Bacteriol.* 144:698; Harlander (1987) "Transformation of *Streptococcus lactis* by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* 32:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* 54:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* 1:412, *Streptococcus*].

#### v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:1].

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes,

combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff *et al.* (1981) *Nature* 283:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* 11:163; Panthier *et al.* (1980) *Curr. Genet.* 2:109;].

- 10 A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.
- 15 Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be
- 20 linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *eg.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method,
- 25 therefore, native foreign protein can be isolated (*eg.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The

leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (US  
5 patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino  
10 acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (eg. see WO 89/02463.)

15 Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

20 Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast  
25 for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al.* (1979) *Gene* 8:17-24], pCl/1 [Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA* 81:4642-4646], and YRp17 [Stinchcomb *et al.* (1982) *J. Mol. Biol.* 158:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and

usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *eg. Brake et al., supra*.

- 5 Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in*  
10 *Enzymol.* 101:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al., supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA* 80:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results  
15 in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may  
20 include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol.*  
25 *Rev.* 51:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts: *Candida albicans* [Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142], *Candida maltosa* [Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141], *Hansenula polymorpha* [Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302], *Kluyveromyces fragilis* [Das, *et al.* (1984) *J. Bacteriol.* 158:1165], *Kluyveromyces lactis* [De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135], *Pichia guillerimondii* [Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141], *Pichia pastoris* [Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; US Patent Nos. 4,837,148 and 4,929,555], *Saccharomyces cerevisiae* [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163], *Schizosaccharomyces pombe* [Beach and Nurse (1981) *Nature* 300:706], and *Yarrowia lipolytica* [Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *eg.* [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; *Candida*]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; *Hansenula*]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; *Kluyveromyces*]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; US Patent Nos. 4,837,148 and 4,929,555; *Pichia*]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 *Saccharomyces*]; [Beach and Nurse (1981) *Nature* 300:706; *Schizosaccharomyces*]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; *Yarrowia*].

### Antibodies

As used herein, the term “antibody” refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An “antibody combining site” is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. “Antibody”

includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying Neisserial proteins.

- 5 Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline,  
10 preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which  
15 for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*eg.* 1,000g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.
- 20 Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [*Nature* (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to  
25 a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*eg.* hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution,  
30 and are assayed for the production of antibodies which bind specifically to the immunizing antigen

(and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (eg. in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly  $^{32}\text{P}$  and  $^{125}\text{I}$ ), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example,  $^{125}\text{I}$  may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with  $^{125}\text{I}$ , or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

### Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of

therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

### Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.



Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (eg. see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

### Vaccines

Vaccines according to the invention may either be prophylactic (*ie.* to prevent infection) or therapeutic (*ie.* to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with “pharmaceutically acceptable carriers,” which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents (“adjuvants”). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, etc. pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial

cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (*eg.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*eg.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

- 10 As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

- 15 The immunogenic compositions (*eg.* the immunising antigen/immunogen/polypeptide/protein/nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

- Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.
- 20

- Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg.* nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation,
- 25

and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, *eg.* by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (*eg.* WO98/20734).

- 5 Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

- 10 As an alternative to protein-based vaccines, DNA vaccination may be employed [*eg.* Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

#### Gene Delivery Vehicles

- 15 Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

- 20 The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 25 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses

eg. MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site  
5 from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA  
10 by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant  
15 vector particles. Preferably, the packaging cell lines are made from human parent cells (eg. HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly  
20 preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC No. VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or  
25 isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698,

WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and

Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470.

5 Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional  
10 exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

15 Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and  
20 WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995, WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN  
25 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, *Nature* 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylogach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Trinit virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9,

1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and  
5 in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting  
10 ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex  
15 beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral  
20 delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate  
25 DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods  
30 for gene delivery that can be used for delivery of the coding sequence include, for example, use of



hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, 5 Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will 10 be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

#### Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression 15 of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of 20 administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *eg.* WO93/14778. Examples of cells useful in *ex vivo* applications 25 include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

#### Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

##### A. Polypeptides

One example are polypeptides which include, without limitation: asialoglycoprotein (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

##### B. Hormones, Vitamins, etc.

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

##### C. Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

##### D. Lipids, and Liposomes

The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *eg.* Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See *eg.* Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA*

76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

### E.Lipoproteins

- 5 In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with  
10 the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

- 15 A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

- 20 The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) *Annu Rev. Biochem* 54:699; Law (1986) *Adv. Exp Med. Biol.* 151:162; Chen (1986) *J Biol Chem* 261:12918; Kane (1980) *Proc Natl Acad Sci USA* 77:2465; and Utermann (1984) *Hum Genet* 65:232.

- Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid  
25 content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (*supra*); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, 5 Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* PCT/US97/14465.

#### F. Polycationic Agents

10 Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both *in vitro*, *ex vivo*, and *in vivo* applications. Polycationic agents can 15 be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful 20 as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the 25 list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

### Immunodiagnostic Assays

5 Neisserial antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-Neisserial antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to Neisserial proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and  
10 a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which  
15 are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt  
20 solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

### Nucleic Acid Hybridisation

“Hybridization” refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor  
25 hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.*  
30 [*supra*] Volume 2, chapter 9, pages 9.47 to 9.57.

“Stringency” refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated  $T_m$  of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to  $10^{-9}$  to  $10^{-8}$  g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of  $10^8$  cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than  $10^8$  cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature ( $T_m$ ) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log_{10} C_i) + 0.4[\%(G + C)] - 0.6(\%\text{formamide}) - 600/n - 1.5(\%\text{mismatch}).$$

where  $C_i$  is the salt concentration (monovalent ions) and  $n$  is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

#### Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to “hybridize” with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.



The probe sequence need not be identical to the Neisserial sequence (or its complement) — some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea *et al.* [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated *eg.* backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* [*eg.* see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [*eg.* see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386].

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* [*Meth. Enzymol.* (1987) 155: 335-350]; US patents 4,683,195 and 4,683,202. Two “primer” nucleotides hybridize

with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

- 5 A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the Neisserial sequence (or its complement).
- 10 Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* [*supra*]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected.
- 15 Typically, the probe is labelled with a radioactive moiety.

## BRIEF DESCRIPTION OF THE DRAWINGS

- Figures 1-7** show biochemical data and sequence analysis pertaining to Examples 1, 2, 3, 7, 13, 16 and 19, respectively, with ORFs 40, 38, 44, 52, 114, 41 and 124.. M1 and M2 are molecular weight markers. Arrows indicate the position of the main recombinant product or, in Western blots, the position of the main *N.meningitidis* immunoreactive band. TP indicates *N.meningitidis* total protein extract; OMV indicates *N.meningitidis* outer membrane vesicle preparation. In bactericidal assay results: a diamond (◆) shows preimmune data; a triangle (▲) shows GST control data; a circle (●) shows data with recombinant *N.meningitidis* protein. Computer analyses show a hydrophilicity plot (upper), an antigenic index plot (middle), and an AMPHI analysis (lower).
- 25 AMPHI program has been used to predict T-cell epitopes [Gao *et al.* (1989) *J. Immunol.* 143:3007; Roberts *et al.* (1996) *AIDS Res Hum Retrovir* 12:593; Quakyi *et al.* (1992) *Scand J Immunol* suppl.11:9) and is available in the Protean package of DNASTAR, Inc. (1228 South Park Street, Madison, Wisconsin 53715 USA).

## EXAMPLES

The examples describe nucleic acid sequences which have been identified in *N.meningitidis*, along with their putative translation products. Not all of the nucleic acid sequences are complete *ie.* they encode less than the full-length wild-type protein. It is believed at present that none of the DNA sequences described herein have significant homologs in *N.gonorrhoeae*.

The examples are generally in the following format:

- a nucleotide sequence which has been identified in *N.meningitidis* (strain B)
- the putative translation product of this sequence
- a computer analysis of the translation product based on database comparisons
- a corresponding gene and protein sequence identified in *N.meningitidis* (strain A)
- a description of the characteristics of the proteins which indicates that they might be suitably antigenic
- results of biochemical analysis (expression, purification, ELISA, FACS *etc.*)

The examples typically include details of sequence homology between species and strains. Proteins that are similar in sequence are generally similar in both structure and function, and the homology often indicates a common evolutionary origin. Comparison with sequences of proteins of known function is widely used as a guide for the assignment of putative protein function to a new sequence and has proved particularly useful in whole-genome analyses.

Sequence comparisons were performed at NCBI (<http://www.ncbi.nlm.nih.gov>) using the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [*eg.* see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Searches were performed against the following databases: non-redundant GenBank+EMBL+DDBJ+PDB sequences and non-redundant GenBank CDS translations+PDB+SwissProt+SPupdate+PIR sequences.

Dots within nucleotide sequences (*eg.* position 288 in Example 12) represent nucleotides which have been arbitrarily introduced in order to maintain a reading frame. In the same way, double-underlined nucleotides were removed. Lower case letters (*eg.* position 589 in Example 12) represent ambiguities which arose during alignment of independent sequencing reactions (some of

the nucleotide sequences in the examples are derived from combining the results of two or more experiments).

5 Nucleotide sequences were scanned in all six reading frames to predict the presence of hydrophobic domains using an algorithm based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. These domains represent potential transmembrane regions or hydrophobic leader sequences.

Open reading frames were predicted from fragmented nucleotide sequences using the program ORFFINDER (NCBI).

10 Underlined amino acid sequences indicate possible transmembrane domains or leader sequences in the ORFs, as predicted by the PSORT algorithm (<http://www.psort.nibb.ac.jp>). Functional domains were also predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

15 Various tests can be used to assess the *in vivo* immunogenicity of the proteins identified in the examples. For example, the proteins can be expressed recombinantly and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question *ie.* the protein is an immunogen. This method can also be used to identify immunodominant proteins.

20 The recombinant protein can also be conveniently used to prepare antibodies *eg.* in a mouse. These can be used for direct confirmation that a protein is located on the cell-surface. Labelled antibody (*eg.* fluorescent labelling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein.

In particular, the following methods (A) to (S) were used to express, purify and biochemically characterise the proteins of the invention:

#### **A) Chromosomal DNA preparation**

25 *N.meningitidis* strain 2996 was grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% Sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50µg/ml Proteinase K), and the suspension was incubated at 37°C for 2

hours. Two phenol extractions (equilibrated to pH 8) and one  $\text{ChCl}_3$ /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4ml buffer (10mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

## B) Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus as necessary. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

The 5' primers included two restriction enzyme recognition sites (*Bam*HI-*Nde*I, *Bam*HI-*Nhe*I, or *Eco*RI-*Nhe*I, depending on the gene's own restriction pattern); the 3' primers included a *Xho*I restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *Bam*HI-*Xho*I or *Eco*RI-*Xho*I), and pET21b+ (using either *Nde*I-*Xho*I or *Nhe*I-*Xho*I).

5'-end primer tail: CGCGGATCCCATATG (*Bam*HI-*Nde*I)

CGCGGATCCGCTAGC (*Bam*HI-*Nhe*I)

CCGGAATTCTAGCTAGC (*Eco*RI-*Nhe*I)

3'-end primer tail: CCCGCTCGAG (*Xho*I)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridised to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$$T_m = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_m = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

**Table I** shows the forward and reverse primers used for each amplification. Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2ml  $\text{NH}_4\text{OH}$ , and deprotected by 5 hours incubation at  $56^\circ\text{C}$ . The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100 $\mu\text{l}$  or 1ml of water.  $\text{OD}_{260}$  was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10pmol/ $\mu\text{l}$ .

### C) Amplification

The standard PCR protocol was as follows: 50-200ng of genomic DNA were used as a template in the presence of 20-40 $\mu\text{M}$  of each oligo, 400-800 $\mu\text{M}$  dNTPs solution, 1x PCR buffer (including 1.5mM  $\text{MgCl}_2$ ), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10 $\mu\text{l}$  DMSO or 50 $\mu\text{l}$  2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at  $95^\circ\text{C}$ ), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at  $72^\circ\text{C}$ .

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds $95^\circ\text{C}$	30 seconds $50-55^\circ\text{C}$	30-60 seconds $72^\circ\text{C}$
Last 30 cycles	30 seconds $95^\circ\text{C}$	30 seconds $65-70^\circ\text{C}$	30-60 seconds $72^\circ\text{C}$

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

#### **D) Digestion of PCR fragments**

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

- *NdeI/XhoI* or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion
- *BamHI/XhoI* or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as N-terminus GST fusion.
- *EcoRI/PstI*, *EcoRI/Sall*, *Sall/PstI* for cloning into pGex-His and further expression of the protein as N-terminus His-tag fusion

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs ) in a either 30 or 40µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 or 50µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

#### **E) Digestion of the cloning vectors (pET22B, pGEX-KG, pTRC-His A, and pGex-His)**

10µg plasmid was double-digested with 50 units of each restriction enzyme in 200µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the

whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50µl of 10mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD<sub>260</sub> of the sample, and adjusted to 50µg/µl. 1µl of plasmid was used for each cloning procedure.

- 5 The vector pGEX-His is a modified pGEX-2T vector carrying a region encoding six histidine residues upstream to the thrombin cleavage site and containing the multiple cloning site of the vector pTRC99 (Pharmacia).

### F) Cloning

- 10 The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

- 15 In order to introduce the recombinant plasmid in a suitable strain, 100µl *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800µl LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200µl of the supernatant. The suspension was then plated on LB ampicillin (100mg/ml ).

- 20 The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37°C in either 2ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100µg/ml ampicillin. The cells were then pelleted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30µl. 5µl of each individual miniprep (approximately 1g ) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.
- 25



### G) Expression

Each ORF cloned into the expression vector was transformed into the strain suitable for expression of the recombinant protein product. 1µl of each construct was used to transform 30µl of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described  
5 above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 100ml flasks, making sure that the OD<sub>600</sub> ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for  
10 induction of expression (0.4-0.8 OD for pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge,  
15 the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000g and the pellet resuspended in PBS for further use.

### H) GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were  
20 diluted 1:30 into 600ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed twice and centrifuged again.  
25 The supernatant was collected and mixed with 150µl Glutathione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD<sub>280</sub> of 0.02-0.06. The GST-fusion  
30 protein was eluted by addition of 700µl cold Glutathione elution buffer (10mM reduced

glutathione, 50mM Tris-HCl) and fractions collected until the OD<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M2) (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

#### **I) His-fusion solubility analysis**

To analyse the solubility of the His-fusion expression products, pellets of 3ml cultures were resuspended in buffer M1 [500µl PBS pH 7.2]. 25µl lysozyme (10mg/ml) was added and the bacteria were incubated for 15 min at 4°C. The pellets were sonicated for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed twice and then separated again into pellet and supernatant by a centrifugation step. The supernatant was collected and the pellet was resuspended in buffer M2 [8M urea, 0.5M NaCl, 20mM imidazole and 0.1M NaH<sub>2</sub>PO<sub>4</sub>] and incubated for 3 to 4 hours at 4°C. After centrifugation, the supernatant was collected and the pellet was resuspended in buffer M3 [6M guanidinium-HCl, 0.5M NaCl, 20mM imidazole and 0.1M NaH<sub>2</sub>PO<sub>4</sub>] overnight at 4°C. The supernatants from all steps were analysed by SDS-PAGE.

#### **J) His-fusion large-scale purification.**

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD<sub>550</sub> 0.6-0.8. Protein expression was induced by addition of 1mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml of either (i) cold buffer A (300mM NaCl, 50mM phosphate buffer, 10mM imidazole, pH 8) for soluble proteins or (ii) buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8) for insoluble proteins.

The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again.

For insoluble proteins, the supernatant was stored at -20°C, while the pellets were resuspended in 2ml buffer C (6M guanidine hydrochloride, 100mM phosphate buffer, 10mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000rpm for 40 minutes.

Supernatants were collected and mixed with 150µl Ni<sup>2+</sup>-resin (Pharmacia) (previously washed with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700g for 5 minutes at 4°C. The resin was washed twice with 10ml buffer A or B for 10 minutes, resuspended in 1ml buffer A or B and loaded on a disposable column. The resin was washed at either (i) 4°C with 2ml cold buffer A or (ii) room temperature with 2ml buffer B, until the flow-through reached OD<sub>280</sub> of 0.02-0.06.

The resin was washed with either (i) 2ml cold 20mM imidazole buffer (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8) or (ii) buffer D (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the O.D<sub>280</sub> of 0.02-0.06. The His-fusion protein was eluted by addition of 700µl of either (i) cold elution buffer A (300mM NaCl, 50mM phosphate buffer, 250mM imidazole, pH 8) or (ii) elution buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the O.D<sub>280</sub> was 0.1. 21µl of each fraction were loaded on a 12% SDS gel.

#### **K) His-fusion proteins renaturation**

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20µg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

#### **L) His-fusion large-scale purification**

500ml of bacterial cultures were induced and the fusion proteins were obtained soluble in buffer M1, M2 or M3 using the procedure described above. The crude extract of the bacteria was loaded

onto a Ni-NTA superflow column (Qiagen) equilibrated with buffer M1, M2 or M3 depending on the solubilization buffer of the fusion proteins. Unbound material was eluted by washing the column with the same buffer. The specific protein was eluted with the corresponding buffer containing 500mM imidazole and dialysed against the corresponding buffer without imidazole.

- 5 After each run the columns were sanitized by washing with at least two column volumes of 0.5 M sodium hydroxide and reequilibrated before the next use.

#### **M) Mice immunisations**

- 20µg of each purified protein were used to immunise mice intraperitoneally. In the case of ORF 44, CD1 mice were immunised with Al(OH)<sub>3</sub> as adjuvant on days 1, 21 and 42, and immune response  
10 was monitored in samples taken on day 56. For ORF 40, CD1 mice were immunised using Freund's adjuvant, rather than Al(OH)<sub>3</sub>, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for ORF 38, CD1 mice were immunised with Freund's adjuvant, but the immune response was measured on day 49.

#### **N) ELISA assay (sera analysis)**

- 15 The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000rpm. The  
20 supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200µl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in  
25 water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at

37°C. Wells were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H<sub>2</sub>O) were added to each well and the plates were left at room temperature for 20 minutes. 100µl H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA was considered positive when OD<sub>490</sub> was 2.5 times the respective pre-immune sera.

#### O) FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN<sub>3</sub>) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD<sub>620</sub> of 0.07. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)<sub>2</sub> goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H threshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539; compensation values: 0.

#### P) OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10 minutes on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000g for 10 minutes and the total cell envelope fraction recovered by centrifugation

at 50000g at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

### **Q) Whole Extracts preparation**

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes.

### **R) Western blotting**

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

### **S) Bactericidal assay**

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD<sub>620</sub> was 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD<sub>620</sub> of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted mice sera (1:100 in Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1 hour were counted.

10 **Table II** gives a summary of the cloning, expression and purification results.

### Example 1

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 1>:

```

15      1  ..ACACTGTTGT TTGCAACGGT TCAGGCAAGT GCTAACCAAT GAAGAGCAAG
      51  AAGAAGATTT ATATTTAGAC CCCGTACAAC GCACTGTTGC CGTGTTGATA
      101  GTCAATTCCG ATAAAGAAGG CACGGGAGAA AAAGAAAAAG TAGAAGAAAA
      151  TTCAGATTGG GCAGTATATT TCAACGAGAA AGGAGTACTA ACAGCCAGAG
      201  AAATCACCyT CAAAGCCGGC GACAACCTGA AAATCAAACA AAACGGCACA
      251  AACTTCACCT ACTCGCTGAA AAAAGACCTC AcAGATCTGA CCAGTGTGG
      301  AACTGAAAAA TTATCGTTTA GCGCAAACGG CAATAAAGTC AACATcACAA
20      351  GCGACACCAA AGGCTTGAAT TTTGCGAAAG AAACGGCTGG sACGAACGgC
      401  GACACCACGG TTCATCTGAA CGGTATTGGT TCGACTTTGA CCGATACGCT
      451  GCTGAATACC GGAGCGACCA CAAACGTAAC CAACGACAAC GTTACCGATG
      501  ACGAGAAAAA ACGTGGCGCA AGCGTTAAAG ACGTATTAAA CGCTGCTGG
      551  AACATTAAAG GCGTTAAACC CGGTACAACA GCTTCCGATA ACGTTGATTT
25      601  CGTCCGCACT TACGACACAG TCGAGTTCTT GAGCGCAGAT ACGAAAACAA
      651  CGACTGTAA TGTGGAAAGC AAAGACAACG GCAAGAAAAC CGAAGTTAAA
      701  ATCGGTGCGA AGACTTCTGT TATTAAAGAA AAAGAC...
```

This corresponds to the amino acid sequence <SEQ ID 2; ORF40>:

```

30      1  ..TLLFATVQAS ANQEEQEEDL YLDPVQRTVA VLIVNSDKEG TGEKEKVEEN
      51  SDWAVYFNEK GVLTAAREITX KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG
      101  TEKLSFSANG NKVNITSDTK GLNFAKETAG TNGDTTVHLN GIGSTLDTL
      151  LNTGATTNVT NDNVTDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF
      201  VRTYDTVEFL SADTKTTTVN VESKDNKKKT EVKIGAKTSV IKEKD...
```

Further work revealed the complete DNA sequence <SEQ ID 3>:

```

35      1  ATGAACAAAA TATACCGCAT CATTGGAAT AGTGCCCTCA ATGCCTGGGT
      51  CGTCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG
      101  TGAAGACCGC CGTATTGGCG ACACTGTTGT TTGCAACGGT TCAGGCAAGT
      151  GCTAACAAATG AAGAGCAAGA AGAAGATTTA TATTTAGACC CCGTACAACG
      201  CACTGTTGCC GTGTTGATAG TCAATTCCGA TAAAGAAGGC ACGGGAGAAA
40      251  AAGAAAAAGT AGAAGAAAAT TCAGATTGGG CAGTATATTT CAACGAGAAA
      301  GGAGTACTAA CAGCCAGGGA AATCACCCCTC AAAGCCGGCG ACAACCTGAA
      351  AATCAAACAA AACGGCACAA ACTTCACCTA CTCGCTGAAA AAAGACCTCA
      401  CAGATCTGAC CAGTGTGGA ACTGAAAAAT TATCGTTTAG CGCAAACGGC
      451  AATAAAGTCA ACATCACAAAG CGACACCAA GGCTTGAATT TTGCGAAAGA
45      501  AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATTGGTT
      551  CGACTTTGAC CGATACGCTG CTGAATACCG GAGCGACCAC AAACGTAACC
      601  AACGACAACG TTACCGATGA CGAGAAAAAA CGTGGCGCAA GCGTTAAAGA
```

651 CGTATTAAAC GCTGGCTGGA ACATTAAAGG CGTTAAACCC GGTACAACAG  
 701 CTTCCGATAA CGTTGATTTT GTCCGCACTT ACGACACAGT CGAGTTCTTG  
 751 AGCGCAGATA CGAAAACAAC GACTGTTAAT GTGGAAAGCA AAGACAACGG  
 801 CAAGAAAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA  
 851 AAGACGGTAA GTTGGTTACT GGTAAAGACA AAGGCGAGAA TGGTTCTTCT  
 901 ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT  
 951 AAACAAGGCT GGTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG  
 1001 GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT  
 1051 GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA  
 1101 CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC  
 1151 AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT  
 1201 TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA  
 1251 TGAAACCGTC AACATTAATG CCGGCAACAA CATCGAGATT ACCCGCAACG  
 1301 GTAAAAATAT CGACATCGCC ACTTCGATGA CCGCGCAGTT TTCCAGCGTT  
 1351 TCGCTCGGCG CGGGGGCGGA TGCGCCCACT TTGAGCGTGG ATGGGGACGC  
 1401 ATTGAATGTC GGCAGCAAGA AGGACAACAA ACCCGTCCGC ATTACCAATG  
 1451 TCGCCCCGGG CGTTAAAGAG GGGGATGTTA CAAACGTCGC ACAACTTAAA  
 1501 GCGTGGCGC AAAACTTGAA CAACCGCATC GACAATGTGG ACGGCAACGC  
 1551 GCGTGGGGC ATCGCCCAAG CGATTGCAAC CGCAGGTCTG GTTCAGGCGT  
 1601 ATTTGCCCGG CAAGATATG ATGGCGATCG GCGGCGGCAC TTATCGGCGC  
 1651 GAAGCCGGTT ACGCCATCGG CTACTCCAGT ATTTCCGACG GCGGAAATTG  
 1701 GATTATCAAA GGCACGGCTT CCGGCAATTC GCGCGGCCAT TTCGGTGCTT  
 1751 CCGCATCTGT CGGTTATCAG TGGTAA

This corresponds to the amino acid sequence <SEQ ID 4; ORF40-1>:

1 MNKIYRIWN SALNAWVVVS ELTRNHTKRA SATVKTAVLA TLLFATVQAS  
 51 ANNEEQEEDL YLDPVQRTVA VLIIVNSDKEG TGEKEKVEEN SDWAVYFNEK  
 101 GVLTAAREITL KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG TEKLSFSANG  
 151 NKVNITSDTK GLNFAKETAG TNGDFTVHLN GIGSTLTDLT LNTGATTNVT  
 201 NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF VRTYDVEFL  
 251 SADTKTTTVN VESKDNKKK EVKIGAKTSV IKEKDGLVT GKDKGENGSS  
 301 TDEGEGLVTA KEVIDAVNKA GWRMKTITAN GQTGQADKFE TVTSGTNVTF  
 351 ASGKGTTATV SKDDQGNITV MYDVNVGDAL NVNQLQNSGW NLDSKAVAGS  
 401 SGKVISGNVS PSKGKMDETV NINAGNNIEI TRNGKNIDIA TSMTPQFSSV  
 451 SLGAGADAPT LSVVDGDLNV GSKKDNKPV ITNVAPGVKE GDVTNVAQLK  
 501 GVAQNLNNRI DNVGDNARAG IAQAIATAGL VQAYLPGKSM MAIGGGTYRG  
 551 EAGYAIGYSS ISDGGNWI IK GTASGNSRGH FGASASVGYQ W\*

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 5 >:

1 ATGAACAAAA TATACCGCAT CATTGGAAT AGTGCCCTCA ATGCCTGNGT  
 51 CGCCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG  
 101 TGAAGACCGC CGTATTGGCG AACTGTTGT TTGCAACGGT TCAGGCGAAT  
 151 GCTACCGATG AAGATGAAGA AGAAGAGTTA GAATCCGTAC AACGCTCTGT  
 201 CGTAGGGAGC ATCAAGCCA GTATGGAAGG CAGCGGCGAA TTGGAACGA  
 251 TATCATTATC AATGACTAAC GACAGCAAGG AATTTGTAGA CCCATACATA  
 301 GTAGTTACCC TCAAAGCCGG CGACAACCTG AAAATCAAAC AAAACACCAA  
 351 TGAAAACACC AATGCCAGTA GCTTCACCTA CTCGCTGAAA AAAGACCTCA  
 401 CAGGCCTGAT CAATGTTGAN ACTGAAAAAT TATCGTTTGG CGCAAACGGC  
 451 AAGAAAGTCA ACATCATAAG CGACACCAA GGCTTGAATT TCGCGAAGA  
 501 AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATCGGTT  
 551 CGACTTTGAC CGATACGCTT GCGGGTTCTT CTGCTTCTCA CGTTGATGCG  
 601 GGTAACCNA GTACACATTA CACTCGTGCA GCAAGTATTA AGGATGTGTT  
 651 GAATGCGGGT TGGAATATTA AGGGTGTTAA ANNGGCTCA ACACTGGTC  
 701 AATCAGAAAA TGTCGATTTT GTCCGCACTT ACGACACAGT CGAGTTCTTG  
 751 AGCGCAGATA CGNAAACAAC GACNGTTAAT GTGGAAAGCA AAGACAACGG  
 801 CAAGAGAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA  
 851 AAGACGGTAA GTTGGTTACT GGTAAAGGCA AAGGCGAGAA TGGTTCTTCT  
 901 ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT  
 951 AAACAAGGCT GGTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG  
 1001 GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT  
 1051 GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA  
 1101 CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC  
 1151 AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT  
 1201 TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA  
 1251 TGAAACCGTC AACATTAATG CCGGCAACAA CATCGAGATT AGCCGCAACG  
 1301 GTAAAAATAT CGACATCGCC ACTTCGATGG CGCCGCGAGT TTCCAGCGTT  
 1351 TCGCTCGGCG CGGGGGCAGA TGCGCCCACT TTAAGCGTGG ATGACGAGGG  
 1401 CGCGTTGAAT GTCGGCAGCA AGGATGCCAA CAAACCCGTC CGCATTACCA



5

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1451 ATGTCGCCCC GGGCGTTAAA GANGGGGATG TTACAAACGT CNCACAACTT
1501 AAAGGCGTGG CGCAAAACTT GAACAACCGC ATCGACAATG TGGACGGCAA
1551 CGCGCGTGCN GGCATCGCCC AAGCGATTGC AACCGCAGGT CTGGTTCAGG
1601 CGTATCTGCC CGGCAAGAGT ATGATGGCGA TCGGCGGCGG CACTTATCGC
1651 GGCGAAGCCG GTTACGCCAT CGGCTACTCC AGTATTTCGG ACGGCGGAAA
1701 TTGGATTATC AAAGGCACGG CTTCCGGCAA TTCGCGCGGC CATTTCGGTG
1751 CTTCCGCATC TGTCGGTTAT CAGTGGTAA

```

This encodes a protein having amino acid sequence <SEQ ID 6; ORF40a>:

10

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1 MNKIYRIIWN SALNAXVAVS ELTRNHTKRA SATVKTAVLA TLLFATVQAN
51 ATDEDEEEEL ESVQRSVVG IASMEGSGE LETISLSMTN DSKEFVDPYI
101 VVTLKAGDNL KIKQNTNENT NASSFTYSLK KDLTGLINXV TEKLSFGANG
151 KKVNIISDTK GLNFAKETAG TNGDFTVHLN GIGSTLTDTL AGSSASHVDA
201 GNXSTHYTRA ASIKDVLNAG WNIKGVKXGS TTQSENVDF VRTYDTVEFL
251 SADTXTTTVN VESKDNGKRT EVKIGAKTSV IKEKDGLVLT GKKGENGSS
301 TDEGEGLVTA KEVIDAVNKA GWRMKTITAN GQTGQADKFE TVTSGTNVTF
351 ASGKGTTATV SKDDQGNITV MYDVNVGDAL NVNQLQNSGW NLDSKAVAGS
401 SGKVISGNVS PSKGKMDTV NINAGNNIEI SRNGKNIDIA TSMAPQFSSV
451 SLGAGADAPT LSVDDDEGALN VGSKDANKPV RITNVAPGVK XGDVTNVXQL
501 KGVAQNLLNR IDNV DGNARA GIAQAIATAG LVQAYLPGKS MMAIGGGTYR
551 GEAGYAIGYS SISDGGNWII KGTSAGNSRG HFGASASVGY QW*

```

The originally-identified partial strain B sequence (ORF40) shows 65.7% identity over a 254aa overlap with ORF40a:

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                10      20      30
orf40.pep      TLLFATVQASANQEEQEEEDLYLDPVQRTVA
                |||||:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      SALNAXVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL--ESVQRSV-
                20      30      40      50      60

                40      50      60      70      80
orf40.pep      VLIVNSDKEGTGEKEKVEEN--SDWAVYFNEKGVLTAREITXKAGDNLKIKQN-----GT
                |:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      VGSIQASMEGSGELETISLSMTNDSKEFVDPYIV----VTLKAGDNLKIKQNTNENTNAS
                70      80      90      100     110     120

                90      100     110     120     130     140
orf40.pep      NFTYSLKKDLTDLTSVGTEKLSFANGNKVNITSDTKGLNFAKETAGTNGDFTVHLNGIG
                :|||:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      SFTYSLKKDLTGLINXTEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDFTVHLNGIG
                130     140     150     160     170     180

                150     160     170     180     190     200
orf40.pep      STLTDTLLNTGATTNVTDNDDEKKRAASVKDVLNAGWNIKGVKPGTTA--SDNVDFV
                |||||:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      STLTDTLAGSSAS--HVDAGNXST--HYTRAASIKDVLNAGWNIKGVKXGSTTGQSENVDFV
                190     200     210     220     230     240

                210     220     230     240
orf40.pep      RTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKD
                |||||:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      RTYDTVEFLSADTXTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGLVLTGKKGENGSSST
                250     260     270     280     290     300

```

The complete strain B sequence (ORF40-1) and ORF40a show 83.7% identity in 601 aa overlap:

55

60

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                10      20      30      40      50      60
orf40-1.pep    MNKIYRIIWN SALNAWVVVSELTRNHTKRASATVKTAVLATLLFATVQASANNEEQEEDL
                |||||:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|
orf40a      MNKIYRIIWN SALNAXVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL
                10      20      30      40      50      60

                70      80      90      100     110     119
orf40-1.pep    YLDPVQRTVAVLIVNSDKEGTGEKEKVEEN--SDWAVYFNEKGVLTAREITLKAGDNLKIK
                :|||:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|:::|

```

Homology with Hsf protein encoded by the type b surface fibrils locus of *H.influenzae*  
(accession number U41852)

60	Orf40	1	TLLFATVQASANQEEQEEDLYLDPVQRTVAVLIVNSDXXXXXXXXXXXXXNSDWAVFYNEK	60
	Hsf	41	TLLFATVQA+A E++E LDPV RT VL +SD NS+W +YF+ K	95
65	Orf40	61	GVLTAAREITXKAGDNLKIKQN-----GTNFTYSLKKDLTDLTSVSGTEKLSFSANGNKVN	114
	Hsf	96	GVLKAGAITLKAGDNLKIKONTDESTNASSFTYSLKKDLTDLTSVATEKLSFGANGDKVD	155

Orf40 115 ITSDTKGLNFAKETAGTNGDITVHLNGIGSTLTDTLLNTGAXXXXXXXXXXXXXEKKRAAS 174  
 ITSD GL AK G+ VHLNG+ STL D + NTG EK RAA+  
 5 Hsf 156 ITSDANGLKLAK-----TGNGNVHLNGLDSTLPDAVTNTGVLSSSSFTPNdv-EKTRAAT 209  
 Orf40 175 VKDVLNAGWNIKGKPGTTASDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKI 234  
 VKDVLNAGWNIKG K ++VD V Y+ VEF++ D T V + +K+NGK TEVK  
 10 Hsf 210 VKDVLNAGWNIKGAKTAGGNVESVDLVSAYNNVEFITGDKNTLDVVLTAKENGKTEVKF 269  
 Orf40 235 GAKTSVIKEKD 245  
 KTSVIKEKD  
 Hsf 270 TPKTSVIKEKD 280

ORF40a also shows homology to Hsf:

15 gi|1666683 (U41852) hsf gene product [Haemophilus influenzae] Length = 2353  
 Score = 153 (67.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 Identities = 33/36 (91%), Positives = 34/36 (94%)  
 Query: 16 VAVSELTRNHTKRASATVKTAVLATLLFATVQANAT 51  
 V VSELTR HTKRASATV+TAVLATLLFATVQANAT  
 20 Sbjct: 17 VVVSELTRTHTKRASATVETAVLATLLFATVQANAT 52  
 Score = 161 (71.2 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 Identities = 32/38 (84%), Positives = 36/38 (94%)  
 25 Query: 101 VTLKAGDNLKIKQNTNENTNASSFTYSLKKDLTGLINV 138  
 +TLKAGDNLKIKQNT+E+TNASSFTYSLKKDLT L +V  
 Sbjct: 103 ITLKAGDNLKIKQNTDESTNASSFTYSLKKDLTDLTSV 140  
 Score = 110 (48.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 30 Identities = 21/29 (72%), Positives = 25/29 (86%)  
 Query: 138 VTEKLSFGANGKKVNIISDTKGLNFAKET 166  
 V++KLS G NG KVNI SDTKGLNFAK++  
 35 Sbjct: 1439 VSDKLSLGTNGNKVNITSDTKGLNFAKDS 1467  
 Score = 85 (37.6 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 Identities = 18/32 (56%), Positives = 20/32 (62%)  
 40 Query: 169 TNGDITVHLNGIGSTLTDTLAGSSASHVDAGN 200  
 T D +HLNGI STLDTL S A+ GN  
 Sbjct: 1469 TGDDANIHLNGIASTLTDTLLNSGATTNLGGN 1500  
 Score = 92 (40.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 45 Identities = 16/19 (84%), Positives = 19/19 (100%)  
 Query: 206 RAASIKDVLNAGWNIKGK 224  
 RAAS+KDVLNAGWN++GK  
 50 Sbjct: 1509 RAASVKDVLNAGWNVRGK 1527  
 Score = 90 (39.8 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116  
 Identities = 17/28 (60%), Positives = 20/28 (71%)  
 Query: 226 STTGQSENVDFVRTYDTVEFLSADTTTT 253  
 S Q EN+DFV TYDTV+F+S D TT  
 55 Sbjct: 1530 SANNQVENIDFVATYDTVDFVSGDKDTT 1557

Based on homology with Hsf, it was predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

ORF40-1 (61kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure  
 60 1A shows the results of affinity purification of the His-fusion protein, and Figure 1B shows the

results of expression of the GST-fusion in *E. coli*. Purified His-fusion protein was used to immunise mice, whose sera were used for FACS analysis (Figure 1C), a bactericidal assay (Figure 1D), and ELISA (positive result). These experiments confirm that ORF40-1 is a surface-exposed protein, and that it is a useful immunogen.

5 Figure 1E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF40-1.

## Example 2

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 7>

```

1  ATGTTACGTT  TGA CTGCTTT  AGCCGTATGC  ACCGCCCTCG  CTTTGGGCGC
10  51  GTGTTGCGCG  CAAAATTCCG  ACTCTGCCCC  ACAAGCCAAA  GaACAGGCGG
101  TTTCCGCGCG  ACAAACCGAA  GgCGCGTCCG  TTACCGTCAA  AACCGCGCGC
151  GGCGACGTTT  AAATACCGCA  AAACCCCGAA  CGCATCGCCG  TTTACGATTT
201  GGGTATGCTC  GACACCTTGA  GCAAACCTGG  CGTGAAAACC  GGTTTGTCCG
251  TCGATAAAAA  CCGCTGCGG  TATTTAGAGG  AATATTTCAA  AACGACAAAA
301  CCTGCGGCA  CTTTGTTCGA  GCCGGATTAC  GAAACGCTCA  ACGCTTACAA
15  351  ACCGCAGCTC  ATCATCATCG  GCAGCCGCGC  CgCCAAGGCG  TTTGACAAAT
401  TGAACGAAAT  CGCGCCGACC  ATCGrmwTGA  CCGCCGATAC  CGCCAACCTC
451  AAAGAAAGTG  CCAArGAGGC  ATCGACGCTG  GCGCAAATCT  TC..

```

This corresponds to the amino acid sequence <SEQ ID 8; ORF38>:

```

20  1  MLRLTALAVC  TALALGACSP  QNSDSAPQAK  EQAVSAAQTE  GASVTVKTAR
51  GDVQIPQNPE  RIAVYDLGML  DTLSKLGVKT  GLSVDKNRLP  YLEEFKTTK
101  PAGTLFEPDY  ETLNAYKPQL  IIIGSRAAKA  FDKLNEIAPT  IXXTADTANL
151  KESAKEASTL  AQIF..

```

Further work revealed the complete nucleotide sequence <SEQ ID 9>:

```

25  1  ATGTTACGTT  TGA CTGCTTT  AGCCGTATGC  ACCGCCCTCG  CTTTGGGCGC
51  GTGTTGCGCG  CAAAATTCCG  ACTCTGCCCC  ACAAGCCAAA  GAACAGGCGG
101  TTTCCGCGCG  ACAAACCGAA  GGCGCGTCCG  TTACCGTCAA  AACCGCGCGC
151  GGCGACGTTT  AAATACCGCA  AAACCCCGAA  CGCATCGCCG  TTTACGATTT
201  GGGTATGCTC  GACACCTTGA  GCAAACCTGG  CGTGAAAACC  GGTTTGTCCG
30  251  TCGATAAAAA  CCGCTGCGG  TATTTAGAGG  AATATTTCAA  AACGACAAAA
301  CCTGCGGCA  CTTTGTTCGA  GCCGGATTAC  GAAACGCTCA  ACGCTTACAA
351  ACCGCAGCTC  ATCATCATCG  GCAGCCGCGC  CGCCAAGGCG  TTTGACAAAT
401  TGAACGAAAT  CGCGCCGACC  ATCGAAATGA  CCGCCGATAC  CGCCAACCTC
451  AAAGAAAGTG  CCAAGAGCG  CATCGACGCG  CTGGCGCAA  TCTTCGGCAA
501  ACAGGCGGAA  GCCGACAAGC  TGAAGGCGGA  AATCGACGCG  TCTTTTGAAG
35  551  CCGCGAAAAC  TGCCGCACAA  GGTAAGGGCA  AAGGTTTGGT  GATTTTGGTC
601  AACGGCGGCA  AGATGTCGGC  TTTCCGCCC  TCTTCACGCT  TGGGCGGCTG
651  GCTGCACAAA  GACATCGGCG  TTCCCGCTGT  CGATGAATCA  ATTAAGAAG
701  GCAGCCACGG  TCAGCCTATC  AGCTTTGAAT  ACCTGAAAGA  GAAAAATCCC
751  GACTGGCTGT  TTGTCTTGA  CCGAAGCGCG  GCCATCGGCG  AAGAGGGTCA
40  801  GGCGGCGAAA  GACGTGTTGG  ATAATCCGCT  GGTGCGCGAA  ACAACCGCTT
851  GGAAAAAAGG  ACAGTCTGTG  TACCTCGTTC  CTGAAACTTA  TTTGGCAGCC
901  GGTGGCGCGC  AAGAGCTGCT  GAATGCAAGC  AAACAGGTTG  CCGACGCTTT
951  TAACGCGGCA  AAATAA

```

This corresponds to the amino acid sequence <SEQ ID 10; ORF38-1>:

```

45  1  MLRLTALAVC  TALALGACSP  QNSDSAPQAK  EQAVSAAQTE  GASVTVKTAR
51  GDVQIPQNPE  RIAVYDLGML  DTLSKLGVKT  GLSVDKNRLP  YLEEFKTTK
101  PAGTLFEPDY  ETLNAYKPQL  IIIGSRAAKA  FDKLNEIAPT  IEMTADTANL
151  KESAKERIDA  LAQIFGKQAE  ADKLKAEIDA  SFEAAKTAAQ  GKKGKLVILV
201  NGGKMSAFGP  SSRLGWLHK  DIGVPAVDES  IKEGSHGQPI  SFEYLKEKNP
50  251  DWLFVLDRSA  AIGEEGQAAK  DVLNPLVAE  TTAWKKGVV  YLVPETYLAA

```

301 GGAQELLNAS KQVADAFNAA K\*

Computer analysis of this amino acid sequence reveals a putative prokaryotic membrane lipoprotein lipid attachment site (underlined).

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 11>:

```

5      1  ATGTTACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC
      51  GTGTTCCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GAACAGGCGG
     101  TTTCCGCCCGC ACAATCCGAA GGCGTGTCG TTACCGTCAA AACGGCGCGC
     151  GGCGATGTTT AAATACCGCA AAACCCCGAA CGTATCGCCG TTTACGATTT
     201  GGGTATGCTC GACACCTTGA GCAAACCTGG CGTGAAAACC GGTGTGTCG
10    251  TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA
     301  CCTGCCGGAA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA
     351  ACCGCAGCTC ATCATCATCG GCAGCCGCGC AGCCAAAGCG TTTGACAAAT
     401  TGAACGAAAT CGCGCCGACC ATCGAAATGA CCGCCGATAC CGCCAACCTC
     451  AAAGAAAGTG CCAAAGAGCG TATCGACGCG CTGGCGCAAA TCTTCGGCAA
15    501  AAAGGCGGAA GCCGACAAGC TGAAGGCGGA AATCGACGCG TCTTTTGAAG
     551  CCGCGAAAAC TGCCGCGCAA GGCAAAGGCA AGGGTTTGGT GATTTTGGTC
     601  AACGGCGGCA AGATGTCCGC CTTCCGCCCG TCTTCACGAC TGGGCGGCTG
     651  GCTGCACAAA GACATCGGCG TTCCCGCTGT TGACGAAGCC ATCAAAGAAG
20    701  GCAGCCACGG TCAGCCTATC AGCTTTGAAT ACCTGAAAGA GAAAAATCCC
     751  GACTGGCTGT TTGTCTTGA CCGCAGCGCG GCCATCGGCG AAGAGGGTCA
     801  GCGCGCGAAA GACGTGTTGA ACAATCCGCT GGTGCGCGAA ACAACCGCTT
     851  GGAAAAAAGG ACAAGTCGTT TACCTTGTTT CTGAAACTTA TTTGGCAGCC
     901  GGTGGCGCGC AAGAGCTACT GAATGCAAGC AAACAGGTTG CCGACGCTTT
     951  TAACGCGGCA AAATAA

```

25 This encodes a protein having amino acid sequence <SEQ ID 12; ORF38a>:

```

      1  MLRLTALAVC TALALGACSP QNSDSAPQAK EQAVSAAQSE GVSVTVK TAR
     51  GDVQIPQNP RIADV DLGML DTL SKLGVKT GLSVDKNR LP YLEEFKTTK
    101  PAGTLFEPDY ETLNAYKPQL IIIGSRAAKA FDKLNEIAPT IEMTADTANL
    151  KESAKERIDA LAQIFGKKAE ADKLKAEIDA SFEAAKTA AQ GKGLVILV
    201  NKGKMSAFGP SSRLGGWLHK DIGVPAVDEA IKEGSHGQPI SFEYLKEKNP
    251  DWLFVLD RSA AIGEEGQAAK DVLNNPLVAE TTAWKKGQV YLVPETYLA A
    301  GGAQELLNAS KQVADAFNAA K*

```

The originally-identified partial strain B sequence (ORF38) shows 95.2% identity over a 165aa overlap with ORF38a:

```

35      orf38.pep      10      20      30      40      50      60
      MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTARGDVQIPQNP
      orf38a          10      20      30      40      50      60
      MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQSEGVSVTVK TARGDVQIPQNP

40      orf38.pep      70      80      90      100     110     120
      RIAVYDLGMLDTLSKLGVTGLSVDKNR LPYLEEYFKTTK PAGTLFEPDYETLNAYKPQL
      orf38a          70      80      90      100     110     120
      RIAVYDLGMLDTLSKLGVTGLSVDKNR LPYLEEYFKTTK PAGTLFEPDYETLNAYKPQL

45      orf38.pep      130     140     150     160
      IIIGSRAAKAFDKLNEIAPTIXXTADTANLKESAKE-ASTLAQIF
      orf38a          130     140     150     160     170     180
      IIIGSRAAKAFDKLNEIAPT IEMTADTANLKESAKERIDALAQIFGKKAEADKLKAEIDA

50      orf38a          190     200     210     220     230     240
      SFEAAKTA AQGKGLVILVNGGKMSAFGPSRLGGWLHKDIGVPAVDEAIKEGSHGQPI

```

55 The complete strain B sequence (ORF38-1) and ORF38a show 98.4% identity in 321 aa overlap:

```

    orf38a.pep      MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQSEGVSVTVKTARGDVQIPQNPE
    orf38-1         MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTARGDVQIPQNPE
5   orf38a.pep      RIAVYDLGMLDRTLKGLGKVTGLSVDKNRLPYLEEFKTTKPGATLFEPTYETLNAYKPQL
    orf38-1         RIAVYDLGMLDRTLKGLGKVTGLSVDKNRLPYLEEFKTTKPGATLFEPTYETLNAYKPQL
10  orf38a.pep      IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKKAEADKLKAEIDA
    orf38-1         IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKQAEADKLKAEIDA
15  orf38a.pep      SFEAAKTAAQGGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDEAIKEGSHGQPI
    orf38-1         SFEAAKTAAQGGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDESIKEGSHGQPI
20  orf38a.pep      SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLDNPLVAETTAWKKGQVVYLVPEYTLAA
    orf38-1         SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLDNPLVAETTAWKKGQVVYLVPEYTLAA
    orf38a.pep      GGAQELLNASKQVADAFNAAK
    orf38-1         GGAQELLNASKQVADAFNAAK

```

Computer analysis of these sequences revealed the following:

#### 25 Homology with a lipoprotein (lipo) of *C.jejuni* (accession number X82427)

ORF38 and lipo show 38% aa identity in 96 aa overlap:

```

    Orf38: 40  EGASVTVKTARGDVQIPQNPERIAVYDLGMLDRTLKGLGKVTGLS-VDKNRLPYLEEFK 98
               EG S  VK + G+ + P+NP ++ + DLG+LDT  L +  ++ V  LP  + FK
30  Lipo:  51  EGDSFLVKDSLGENKTPKNPSKVILDLDLGILDTFDALKLNDKVAGVPAKNLPKYLQQFKN 110
    Orf38: 99  TKPGATLFEPTYETLNAYKPQLIIIGSRAAKAFDKL 134
               G + + D+E +NA KP LIII  R +K +DKL
    Lipo: 111  KPSVGGVQQVDFAINALKPDLIISGRQSKFYDKL 146

```

Based on this analysis, it was predicted that this protein from *N.meningitidis*, and its epitopes, could  
 35 be useful antigens for vaccines or diagnostics.

ORF38-1 (32kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 2A shows the results of affinity purification of the His-fusion protein, and Figure 2B shows the results of expression of the GST-fusion in *E.coli*. Purified His-fusion protein was used to immunise  
 40 mice, whose sera were used for Western blot analysis (Figure 2C) and FACS analysis (Figure 2D). These experiments confirm that ORF38-1 is a surface-exposed protein, and that it is a useful immunogen.

Figure 2E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF38-1.

#### Example 3

45 The following *N.meningitidis* DNA sequence was identified <SEQ ID 13>:

5

```

1  ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG
51 TATGGCTGCC GCCGCTGGCA CGGACAACCC CACTGTTGCA AAAAAAACCG
101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTC
151 AACAAACAGG GTCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG
201 CGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT
251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA
301 TCCTACCGCA AACAGCCCAT TATGATTACC GCACCTGACA ACCAAATCGT
351 CTTCAAAGAC TGTTCCCCAC GTTAA

```

This corresponds to the amino acid sequence <SEQ ID 14; ORF44>:

10

```

1  MKLLTTAILS SAIALSSMAA AAGTDNPTVA KKTVSYVCQQ GKVKVITYGF
51  NKQGLTTYAS AVINGKRVQM PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK
101 SYRKQPIMIT APDNQIVFKD CSPR*

```

Computer analysis of this amino acid sequence predicted the leader peptide shown underlined.

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 15>:

15

```

1  ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG
51 TATGGCTGCT GCTGCCGGCA CGAACAACCC CACCGTTGCC AAAAAAACCG
101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTT
151 AACAAACAGG GCCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG
201 TGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT
251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA
301 TCCTATCGCA AACAGCCTAT TATGATTACC GCACCTGACA ACCAAATCGT
351 CTTCAAAGAC TGTTCCCCAC GTTAA

```

20

This encodes a protein having amino acid sequence <SEQ ID 16; ORF44a>:

25

```

1  MKLLTTAILS SAIALSSMAA AAGTNNPTVA KKTVSYVCQQ GKVKVITYGF
51  NKQGLTTYAS AVINGKRVQM PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK
101 SYRKQPIMIT APDNQIVFKD CSPR*

```

The strain B sequence (ORF44) shows 99.2% identity over a 124aa overlap with ORF44a:

```

30  orf44.pep      10      20      30      40      50      60
      MKLLTTAILSSAIALSSMAAAAGTDNPTVAKKTVSYVCQQGKVKVITYGFNKQGLTTYAS
      |||||
      orf44a      MKLLTTAILSSAIALSSMAAAAGTNNPTVAKKTVSYVCQQGKVKVITYGFNKQGLTTYAS
                  10      20      30      40      50      60

35  orf44.pep      70      80      90     100     110     120
      AVINGKRVQMPVNLDKSDNVETFYGKEGGYVLGTGVMDGKSYRKQPIMITAPDNQIVFKD
      |||||
      orf44a      AVINGKRVQMPVNLDKSDNVETFYGKEGGYVLGTGVMDGKSYRKQPIMITAPDNQIVFKD
                  70      80      90     100     110     120

40  orf44.pep      CSPRX
      |||||
      orf44a      CSPRX

```

Computer analysis gave the following results:

Homology with the LecA adhesin of *Eikenella corrodens* (accession number D78153)

45 ORF44 and LecA protein show 45% aa identity in 91 aa overlap:

```

Orf44  33  TVSYVCQQGKVKVITYGFNKQGLTTYASAVINGKRVQMPVNLDKSDNVETFYGKEGGYVL 92
      +V+YVCQQG+++ V Y FN  G+ T A  +N + +++P NL  SDNV+T +   GY L
LecA   135 SVAYVCQQGRRLLNVNRYFNSAGVPTSAELRVNRRNLRLPYNLSASDNVDTVF-SANGYRL 193

50  Orf44  93  GTGVMDGKSYRKQPIMITAPDNQIVFKDCSP 123
      T  MD  +YR Q I+++AP+  Q+++KDCSP

```

LecA 194 TTNAMDSANYRSQDIIVSAPNGQMLYKDCSP 224

Based on homology with the adhesin, it was predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

ORF44-1 (11.2kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 3A shows the results of affinity purification of the His-fusion protein, and Figure 3B shows the results of expression of the GST-fusion in *E.coli*. Purified His-fusion protein was used to immunise mice, whose sera were used for ELISA, which gave positive results, and for a bactericidal assay (Figure 3C). These experiments confirm that ORF44-1 is a surface-exposed protein, and that it is a useful immunogen.

Figure 3D shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF44-1.

#### Example 4

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 17>

```

15      1  ..GGCACCGAAT TCAAAACCAC CTTTCCGGA GCGACATAC AGGCAGGGGT
      51  GGGTGAAAAA GCCCGAGCCG ATGCGAAAAT TATCCTAAAA GGCATCGTTA
     101  ACCGCATCCA AACCGAAGAA AAGCTGGAAT CCAACTCGAC CGTATGGCAA
     151  AAGCAGGCCG GAAGCGGCAG CACGGTTGAA ACGCTGAAGC TACCGAGCTT
     201  TGAAGGGCCG GCACTGCCTA AGCTGACCGC TCCCGGCGGC TATATCGCCG
     251  ACATCCCCAA AGGCACCTC AAAACCGAAA TCGAAAAGCT GGCCAAACAG
     301  CCCGAATATG CCTATCTGAA ACAGCTTCAG ACGGTCAAGG ACGTGAAGTG
     351  GAACCAAGTA CAGCTCGCTT ACGACAAATG GGAATAATA CAGGAAGGCC
     401  TAACCGGAGC CGGAGCCGCA ATTANCGCAC TGGCCGTTAC CGTGGTCACC
     451  TCAGGCGCAG GAACCGGAGC CGTATTGGGA TTAANACGNG TGGCCGCCGC
     501  CGCAACCGAT GCAGCATTT...
```

This corresponds to the amino acid sequence <SEQ ID 18; ORF49>:

```

      1  ..GTEFKTTLTG ADIQAGVGEK ARADAKIILK GIVNRIQTEE KLESNSTVWQ
     51  KQAGSGSTVE TLKLPSFEGP ALPKLTAPGG YIADIPKGNL KTEIEKLAKQ
    101  PEYAYLKQLQ TVKDVNWNQV QLAYDKWDYK QEGLTGAGAA IXALAVTVVT
    151  SGAGTGAVLG LXRVAATAAD AAF..
```

Further work revealed the complete nucleotide sequence <SEQ ID 19>:

```

      1  ATGCAACTGC TGGCAGCCGA AGGCATTAC CAACACCAAT TGAATGTTCA
     51  GAAAAGTACC CGTTTCATCG GCATCAAAGT GGGTAAAAGC AATTACAGCA
    101  AAAACGAGCT GAACGAAACC AAAGTGGCCG TACGCGTTAT CGCCCAAACA
    151  GCCAAAACCC GTTCCGGGTG GGATACCGTA CTCGAAGGCA CCGAATTCAA
    201  AACCACCTT TCCGGAGCCG ACATACAGGC AGGGGTGGGT GAAAAGCCC
    251  GAGCCGATGC GAAAATTATC CTAAAAGGCA TCGTTAACCG CATCCAAACC
    301  GAAGAAAAGC TGGGAATCAA CTCGACCGTA TGGCAAAAGC AGGCCGGAAG
    351  CGGCAGCAGC GTTGAAACGC TGAAGCTACC GAGCTTTGAA GGGCCGGCAC
    401  TGCCTAAGCT GACCGTCCC GCGGCTATA TCGCCGACAT CCCCAAAGGC
    451  AACCTCAAAA CCGAATCGA AAAGCTGGCC AAACAGCCCG AATATGCCTA
    501  TCTGAAACAG CTTTACAGCG TCAAGGACGT GAACTGGAAC CAAGTACAGC
    551  TCGCTTACGA CAAATGGGAC TATAAACAGG AAGGCCTAAC CGGAGCCGGA
    601  GCCGCAATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC
    651  CGGAGCCGTA TTGGGATTAA ACGGTGCGGC CGCCGCCGCA ACCGATGCAG
```



30

35

Computer analysis predicts a transmembrane domain and also indicates that ORF49 has no significant amino acid homology with known proteins. A corresponding ORF from *N.meningitidis* strain A was, however, identified:

ORF49 shows 86.1% identity over a 173aa overlap with an ORF (ORF49a) from strain A of *N. meningitidis*:

45

50

55

[illegible]

```

orf49a      KTEIEKLSKQPEYAYLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAAIIALAVTVVT
              160      170      180      190      200      210
5  orf49.pep      160      170
      SGAGTGAVLGLXRVAATAAFAF
      ||||| : |||||
orf49a      SGAGTGAVLGLNGAXAAATDAAFASLASQASVSFINNKGDVGKTLKELGRSSTVKNLVVA
              220      230      240      250      260      270

```

ORF49-1 and ORF49a show 83.2% identity in 457 aa overlap:

```

10  orf49a.pep      XQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNEINETKLPVRVVAQXAATRSWDTV
      orf49-1      MQLLAEEGIHQHLNVQKSTRFIGIKVGXSNYSKNEINETKLPVRVIAQTAKTRSGWDTV
15  orf49a.pep      LEGTEFKTTLAGADIQAGVXEKARVDAKIIILKGIVNRIQSEEKLETNSTVWQKQAGRGST
      orf49-1      LEGTEFKTTLSGADIQAGVGEKARADAKIIILKGIVNRIQTEEKLESNSTVWQKQAGSGST
      orf49a.pep      IETLKLPSFESPTPPKLSAPGGYIVDIPKGNLKTEIEKLSKQPEYAYLKQLQVAKNINWN
      orf49-1      VETLKLPSFEGPALPKLTAPGGYIADIPKGNLKTEIEKLAKQPEYAYLKQLQTVKDVNWN
20  orf49a.pep      QVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSAGTGAVLGLNGAXAAATDAAFASLAS
      orf49-1      QVQLAYDKWDYKQEGLTGAGAAIIALAVTVVTSAGTGAVLGLNGAAAATDAAFASLAS
25  orf49a.pep      QASVSFINNKGDVGKTLKELGRSSTVKNLVVAATAGVADKIGASALXNVSDKQWINNLT
      orf49-1      QASVSFINNKGNIQNTLKLGRSSTVKNLVAVATAGVADKIGASALNNVSDKQWINNLT
30  orf49a.pep      VNLANAGSAALINTAVNGGSLKDXLEANILAAVNTAHGEAASKIKQLDQHYIVHKIAHA
      orf49-1      VNLANAGSAALINTAVNGGSLKDNLEANILAAVNTAHGEAASKIKQLDQHYIAHKIAHA
35  orf49a.pep      IAGCAAAAANKGKCQDGAIGAAGVEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVS
      orf49-1      IAGCAAAAANKGKCQDGAIGAAGVEILGETLLDGRDPGSLNVKDRAKIIAKAKLAAGAVA
      orf49a.pep      GVVGGDVNAANAEEVAVKNNQLSDXEGREFDNEMTACAKQNXPLCRKNTVKKYQNVAD
      orf49-1      ALSKGDVSTAANAAVAVENNSLNDIQDRLLSGNYALCMSAGGAESFCESYRPLGLPHFV
40  orf49a.pep      KRLAASIAICTDISRSTECRTIRKQHLIDSRSLHSSWEAGLIGKDDEWYKLFKSYTQAD
      orf49-1      SVSGEMKLPNKFGRNMVNGKLIINTRNGNVYFSVGKIWSTVKSTKSNISGVSVGWVNLVS

```

45 The complete length ORF49a nucleotide sequence <SEQ ID 21> is:

```

1  NTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT TGGATGTCCA
51  AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGTNAGAGC AATTACAGTA
101 AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT CGCCCAAANT
50 151 GCAGCCACCC GTTCAGGCTG GGATACCGTG CTCGAAGGTA CCGAATTCAA
201 AACCACGCTG GCCGGTGCCG ACATTACGGC AGGTGTANGC GAAAAAGCCC
251 GTGTCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG TATCCAGTCG
301 GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC AGGCCGGACG
351 CGGCAGCACT ATCGAAACGC TAAAACTGCC CAGCTTCGAA AGCCCTACTC
55 401 CGCCCAAATT GTCCGCACCC GCGGNTATA TCGTCGACAT TCCGAAAGGC
451 AATCTGAAAA CCGAAATCGA AAAGCTGTCC AAACAGCCG AGTATGCCTA
501 TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT CAGGTGCAGC
551 TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC CGAAGCAGGT
601 GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC
651 CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA ACCGATGCAG
60 701 CATTCGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT CAACAACAAA
751 GGCGATGTCG GCAAAACCCT GAAAGAGCTG GGCAGAAGCA GCACGGTGAA
801 AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC AAAATCGGCG
851 CTTCCGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA CAACCTGACC
901 GTCAACCTAG CCAATGCGGG CAGTGCCGCA CTGATTAATA CCGCTGTCAA
65 951 CGGCGGCAGC CTGAAAGACA NTCTGGAAGC GAATATCCTT GCGGCTTTGG
1001 TCAATACCGC GCATGGAGAA GCAGCCAGTA AAATCAAACA GTTGGATCAG

```

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```

1051 CACTACATAG TCCACAAGAT TGCCCATGCC ATAGCGGGCT GTGCGGCAGC
1101 GCGGCGCAAT AAGGGCAAGT GTCAGGATGG TGCGATAGGT GCGGCTGTGG
1151 GCGAGATAGT CGGGGAGGCT TTGACAAACG GCAAAAATCC TGACACTTTG
1201 ACAGCTAAAG AACGCGAACA GATTTTGGCA TACAGCAAAC TGGTTGCCCG
1251 TACGGTAAGC GGTGTGGTCG GCGGCGATGT AAATGCGGCG GCGAATGCGG
1301 CTGAGGTAGC GGTGAAAAAT AATCAGCTTA GCGACNAAGA GGGTAGAGAA
1351 TTTGATAACG AAATGACTGC ATGCGCCAAA CAGAATANTC CTCAACTGTG
1401 CAGAAAAAAT ACTGTAAAAA AGTATCAAAA TGTGTCTGAT AAAAGACTTG
1451 CTGCTTCGAT TGCAATATGT ACGGATATAT CCCGTAGTAC TGAATGTAGA
1501 ACAATCAGAA AACAACATTT GATCGATAGT AGAAGCCTTC ATTCACTCTG
1551 GGAAGCAGGT CTAATTGGTA AAGATGATGA ATGGTATAAA TTATTCAGCA
1601 AATCTTACAC CCAAGCAGAT TTGGCTTTAC AGTCTTATCA TTTGAATACT
1651 GCTGCTAAAT CTTGGCTTCA ATCGGGCAAT ACAAAGCCTT TATCCGAATG
1701 GATGTCGGAC CAAGGTTATA CACTTATTTT AGGAGTTAAT CCTAGATTCA
1751 TTTCAATACC AAGAGGTTT GTAAAACAAA ATACACCTAT TACTAATGTC
1801 AAATACCCGG AAGGCATCAG TTTGATACA AACCTANAAA GACATCTGGC
1851 AAATGCTGAT GGTTTTAGTC AAGAACAGGG CATTAAAGGA GCCCATAACC
1901 GCACCAATNT TATGGCAGAA CTAAATTCAC GAGGAGGANG NGTAAATCT
1951 GAAACCCANA CTGATATTGA AGGCATTACC CGAATTAAAT ATGAGATTCC
2001 TACACTAGAC AGGACAGGTA AACCTGATGG TGGATTTAAG GAAATTTCAA
2051 GTATAAAAAC TGTTTATAAT CCTAAAAANT TTTNNGATGA TAAAATACTT
2101 CAAATGGCTC AANATGCTGN TTCACAAGGA TATTCAAAAG CCTCTAAAAT
2151 TGCTCAAAAT GAAAGAACTA AATCAATATC GGAAAGAAAA AATGTCATTC
2201 AATTCTCAGA AACCTTGAC GGAATCAAAT TTAGANNNTA TNTNGATGTA
2251 AATACAGGAA GAATTACAAA CATTACCCCA GAATAATTTA A

```

This encodes a protein having amino acid sequence <SEQ ID 22>:

30  
35  
40

```

1 XQLLAEEGIH KHELDVQKSR RFIGIKVGXS NYSKNELNET KLPVRVVAQX
51 AATRSWDTV LEGTEFKTTL AGADIQAGVX EKARVDAKII LKGIVNRIQS
101 EEKLETNSTV WQKQAGRGST IETLKLPSFE SPTPPKLSAP GGYIVDIPKG
151 NLKTEIEKLS KQPEYAYLKQ LQVAKNINWN QVQLAYDRWD YKQEGLTEAG
201 AAIIALAVTV VTSGAGTGAV LGLNGAXAAA TDAAFASLAS QASVSFINNK
251 GDVGKTLKEL GRSSTVKNLV VAAATAGVAD KIGASALXNV SDKQWINNLT
301 VNLANAGSAA LINTAVNGGS LKDXLEANIL AALVNTAHGE AASKIKQLDQ
351 HYIVHKIAHA IAGCAAAAAN KGKQDGAIG AAVGEIVGEA LTNGKNPDTL
401 TAKEREQILA YSKLVAGTVS GVVGGDVNAA ANAAEVAVKN NQLSDXEGRE
451 FDNEMTACAK QNXPQLCRKN TVKKYQNVAD KRLAASIAIC TDISRSTECR
501 TIRKQHLIDS RSLHSSWEAG LIGKDDEWYK LFSKSYTQAD LALQSYHLNT
551 AAKSWLQSGN TKPLSEWMSD QGYTLISGVN PRFIPIPRGF VKQNTPIITNV
601 KYPEGISFDT NLXRHLANAD GFSQEQGIKG AHNRTNXMAE LNSRGGXVKS
651 ETXTDIEGIT RIKYEIPTLD RTGKPDGGFK EISSIKTVYN PKXFXDDKIL
701 QMAQXAXSQG YSKASKIAQN ERTKSISERK NVIQFSETFD GIKFRXYXDV
751 NTGRITNIHP E*

```

Based on the presence of a putative transmembrane domain, it is predicted that these proteins from *N.meningitidis*, and their epitopes, could be useful antigens for vaccines or diagnostics.

#### 45 Example 5

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 23>

50

```

1 ..CGGATCGTTG TAGGTTTGGC GATTCTTGC GCCGTAGTCA CCGTAGTCCC
51 AAGTATAACC CAAGGCTTTG TCTTCGCCTT TCATTCCGAT AAGGGATATG
101 ACGCTTTGGT CGGTATAGCC GTCTTGGGAA CCTTTGTCCA CCCAACGCAT
151 ATCTGCCTGC GGATTCTCAT TGCCGCTTCT TGGCTGCTGA TTTTCTGCC
201 TTCGCGTTT TCAACTTCGC GCTTGAGGGC TTCGGCATAT TTGTCGGCCA
251 ACGCCATTTC TTTCGGATGC AGCTGCCTAT TGTTCCAATC TACATTCGCA
301 CCCACCACAG CACCACCACT ACCACCAGTT GCATAG

```

This corresponds to the amino acid sequence <SEQ ID 24; ORF50>:

55

```

1 ..RIVVGLRISC AVVTVVPSIT QGFVFAFHS D KGYDALVGIA VLGT FVHPHTH
51 ICLRILIAAS WLLIFLPSRF STSRLRASAY LSANAISFGC SCLLFQSTFA
101 PTTAPPLPPV A*

```

Computer analysis predicts two transmembrane domains and also indicates that ORF50 has no significant amino acid homology with known proteins.

Based on the presence of a putative transmembrane domain, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

## 5 Example 6

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 25>

```

1  ..AAGTTTGACT TTACCTGGTT TATTCGGGCG GTAATCAAAT ACCGCCGGTT
51  GTTTTTTGAA GTATTGGTGG TGTGGTGGT GTTGCGCTGA TTTGCGCTGA
101 TTACGCCCTCT GTTTTTCCAA GTGGTGATGG ACAAGGTGCT GGTACATCGG
10  151 GGATTCTCTA CTTTGGATGT GGTGTCGGTG GCTTTGTTGG TGGTGTGCGT
201 GTTTGAGATT GTGTTGGGCG GTTTGCGGAC GTATCTGTTT GCACATACGA
251 CTTACAGTAT TGATGTGGAA TTGGGCGCGC GTTTGTTCCG GCATCTGCTT
301 TCCCTGCCTT TATCCTATTT CGAGCACAGA CGAGTGGGTG ATACGGTGGC
15  351 TCGGGTGC GG GAATTGGAGC AGATTGCGAA TTTCTTGACC GGTGAGGCGC
401 TGA CTTCGGT GTTGGATTTG GCGTTTTTCGT TTATCTTTCT GCGCGTGATG
451 TGGTATTACA GCTCCACTCT GACTTGGGTG GTATTGGCTT CGTTG.....
//
1451 .....
1501 ..... ..ATTTGCGC
20  1551 CAACCGGACG GTGCTGATTA TCGCCACCG TCTGTCCACT GTTAAAACGG
1601 CACACCGGAT CATTGCCATG GATAAAGGCA GGATTGTGGA AGCGGGAACA
1651 CAGCAGGAAT TGCTGGCGAA CG..AACGGA TATTACCGCT ATCTGTATGA
1701 TTTACAGAAC GGGTAG

```

This corresponds to the amino acid sequence <SEQ ID 26; ORF39>:

```

25  1  ..KFDFTFWFI PA VIKYRRLFFE VLVVSVVLQL FALITPLFFQ VMDKVLVHR
51  GFSTLDVVS ALLVVSLEFI VLGGLRTRYLF AHTTSRIDVE LGARLFRHLL
101 SLPLSYFEHR RVGDTVARVR ELEQIRNFLT GQALTSVLDL AFSFIFLAVM
151 WYYSSTLTWV VLASL.....
//
30  501 ..... ICA NRT VLI IAHRLST VKTAHRIIAM DKGRIVEAGT
551 QQELLANXNG YYRYLYDLQN G*

```

Further work revealed the complete nucleotide sequence <SEQ ID 27>:

```

1  ATGTCTATCG TATCCGCACC GCTCCCCGCC CTTTCCGCC TCATCATCCT
35  51  CGCCCATTA CACGGCATTG CCGCCAATCC TGCCGATATA CAGCATGAAT
101 TTTGTACTT CACGACAGAG GATTAAATG AAACGCAATG GCTGTTAGCC
151 GCCAAATCTT TGGGATTGAA GGCAAAGGTA GTCCGCCAGC CTATTAAACG
201 TTTGGCTATG GCGACTTTAC CCGCATTGGT ATGGTGTGAT GACGGCAACC
251 ATTTTCATTTT GGCCAAAACA GACGGTGAGG GTGAGCATGC CCAATTTTGT
40  301 ATACAGGATT TGGTTACGAA TAAGTCTGCG GTATTGTCTT TTGCCGAATT
351 TTCTAACAGA TATTGCGGCA AACTGATATT GGTGCTTCC CGCGCTTCGG
401 TATTGGGCAG TTTGGCAAAG TTTGACTTTA CCTGGTTTAT TCCGGCGGTA
451 ATCAAATACC GCCGGTTGTT TTTTGAAGTA TTGGTGGTGT CGGTGGTGT
501 GCAGCTGTTT GCGCTGATTA CGCCTCTGTT TTTCCAAGTG GTGATGGACA
55  551 AGGTGCTGGT ACGTCGGGGA TTCTCTACTT TGGATGTGGT GTCGGTGGCT
601 TTGTTGGTGG TGTGCTGTT TGAGATTGTG TTGGGCGGTT TGCGGACGTA
45  651 TCTGTTTGCA CATACGACTT CACGTATTGA TGTGGAATTG GGCGCGCGTT
701 TGTTCGGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCGA GCACAGACGA
751 GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATT
801 CTTGACCGGT CAGGCGCTGA CTTCCGTGTT GGATTGGCG TTTTCGTTTA
50  851 TCTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA
901 TTGGCTTCGT TGCCTGCCTA TCGGTTTGG TCGGCATTTA TCAGTCCGAT
951 ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCACT
1001 CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCGATGGCG
1051 GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT

```

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```

1101 GGCTTCGGGA TTTCGGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG
1151 TGCAGCTGAT TCAGAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA
1201 CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA
1251 TATGCTCTCG GGACAGGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT
1301 GGCAGGATTT CCAGCAGGTG GGGATTTTCGG TGGCGCGTTT GGGGGATATT
1351 CTGAATGCGC CGACCGAGAA TGGCTCTTCG CATTTGGCTT TGCCCGATAT
1401 CCGGGGGGAG ATTACGTTTC AACATGTCGA TTTCCGCTAT AAGGCGGACG
1451 GCAGGCTGAT TTTGCAGGAT TTGAACCTGC GGATTCGGGC GGGGGAAGTG
1501 CTGGGGGATTG TGGGACGTTT GGGGTTCGGG AAATCCACAC TCACCAAAAT
1551 GGTGCAGCGT CTGTATGTAC CGGAGCAGGG ACGGGTGTGT GTGGACGGCA
1601 ACGATTGGGC TTTGGCCGCT CCTGCCTGGC TGGCGGCGCA GGTGCGCGTG
1651 GTCTTGACAG AGAATGTGCT GCTCAACCGC AGCATACGCG ACAATATCGC
1701 GCTGACGATG ACGGGTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC
1751 TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC
1801 GTGGTGGGCG AACAAGGGGC CGGCTTGTCG GCGCGACAGC GGCAGCGTAT
1851 TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTGTATG
1901 AAGCCACCAG CGCGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC
1951 ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT
2001 GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA
2051 TTGTGGAAGC GGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT
2101 TACCGCTATC TGTATGATT ACAGAACGGG TAG

```

This corresponds to the amino acid sequence <SEQ ID 28; ORF39-1>:

25  
30  
35

```

1 MSIVSAPLPA LSALIILAHY HGIAANPADI QHEFCTSAQS DLNETQWLLA
51 AKSLGLKAKV VRQPIKRLAM ATLPALVWCD DGNHFILAKT DGEGEHAQFL
101 IQDLVTNKSA VLSFAEFSNR YSGKLILVAS RASVLGSLAK FDFTWFIPAV
151 IKYRRLFFEV LVVSVVLQLE ALITPLFFQV VMDKVLVHRG FSTLDVVSVA
201 LLVVSLEFIV LGGLRITYLFA HTTSRIDVEL GARLFRHLLS LPLSYFEHRR
251 VGDTVARVRE LEQIRNFLTQ QALTSVLDLA FSFIFLAVMW YYSSTLTWV
301 LASLPAYAFW SAFISPILRT RLNDKFARNA DNQSFLVESI TAVGTVKAMA
351 VEPQMTQRWD NQLAAYVASG FRVTKLAVVG QQGVQLIQKL VTVATLWIGA
401 RLVIESKLTV GQLIAFNMLS GQVAAPVIRL AQLWQDFQOV GISVARLGDI
451 LNAPTENASS HLALPDIRGE ITFEHVDFRY KADGRLLIQD LNLIRIRAGEV
501 LGIVGRSGSG KSTLTKLVRQ LYVPEQGRVL VDGNDLALAA PAWLRRQVGV
551 VLQENVLLNR SIRDNIALTQ TGMPLERIE AAKLAGAHEF IMELPEGYGT
601 VVGEQGAGLS GGQRQRIATA RALITNPRIL IFDEATSALD YESERAIMQN
651 MQAICANRTV LIIAHLSTV KTAHRIIAMD KGRIVEAGTQ QELLAKPNGY
701 YRYLDLQNG *

```

Computer analysis of this amino acid sequence gave the following results:

#### Homology with a predicted ORF from *N.meningitidis* (strain A)

40 ORF39 shows 100% identity over a 165aa overlap with an ORF (ORF39a) from strain A of *N.meningitidis*:

45  
50  
55  
60

```

              10      20      30
orf39.pep      KFDFTWFIPAVIKYRRLFFFEVLVVSIVLQ
              |||
orf39a      AVLSFAEFSNRYSGLKILVASRASVLGSLAKFDFTWFIPAVIKYRRLFFFEVLVVSIVLQ
              110      120      130      140      150      160

              40      50      60      70      80      90
orf39.pep      FALITPLFFQVMDKVLVHRGFSTLDVVSALLVVSLEFIVLGGLRITYLFAHTTSRIDVE
              |||
orf39a      FALITPLFFQVMDKVLVHRGFSTLDVVSALLVVSLEFIVLGGLRITYLFAHTTSRIDVE
              170      180      190      200      210      220

              100      110      120      130      140      150
orf39.pep      LGARLFRHLLSLPLSYFEHRRVGDTVARVRELEQIRNFLTQALTSVLDLAFSIFLAVM
              |||
orf39a      LGARLFRHLLSLPLSYFEHRRVGDTVARVRELEQIRNFLTQALTSVLDLAFSIFLAVM
              230      240      250      260      270      280

              160      170      180      190      200      210
orf39.pep      WYYSSTLTWVVLASLXXXXXXXXXXXXXXXXXXXXXXXXXICANRTVLIIAHLSTV

```

orf39a                   |||||  
                   WYYSSTLTWVVLASLPAYAFWSAFISPILRTRLNDKFARNADNQSLVESITAVGTVKAM  
                   290               300               310               320               330               340

ORF39-1 and ORF39a show 99.4% identity in 710 aa overlap:

5	orf39-1.pep	MSIVSAPLPALSALIILAHYHGIAANPADIQHEFCTSAQSDLNETQWLLAAKSLGLKAKV
	orf39a	MSIVSAPLPALSALIILAHYHGIAANPADIQHEFCTSAQSDLNETQWLLAAKSLGLKAKV
10	orf39-1.pep	VRQPIKRLAMATLPALVWCDDGNHFILAKTDGEGEHAQFLIQDLVTNKSAVLSFAEFSNR
	orf39a	VRQPIKRLAMATLPALVWCDDGNHFILAKTDGGGEHAQYLIQDLTTNKSAVLSFAEFSNR
15	orf39-1.pep	YSGKLILVASRASVLGSLAKFDFTFWIFPAVIKYRRLFFEVLVSVVLQLFALITPLFFQV
	orf39a	YSGKLILVASRASVLGSLAKFDFTFWIFPAVIKYRRLFFEVLVSVVLQLFALITPLFFQV
20	orf39-1.pep	VMDKVLVHRGFSTLDVVSALLVVSLEIVLGGLRITYLFAHTTSRIDVELGARLFRHLLS
	orf39a	VMDKVLVHRGFSTLDVVSALLVVSLEIVLGGLRITYLFAHTTSRIDVELGARLFRHLLS
25	orf39-1.pep	LPLSYFEHRRVGD TVARVRELEQIRNFLTGQALTSVLDLAFSFI FLAVMWWYSSSTLTWV
	orf39a	LPLSYFEHRRVGD TVARVRELEQIRNFLTGQALTSVLDLAFSFI FLAVMWWYSSSTLTWV
30	orf39-1.pep	NQLAAYVASGFRVTKLAVVGQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLS
	orf39a	NQLAAYVASGFRVTKLAVVGQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLS
35	orf39-1.pep	GQVAAPVIRLAQLWQDFQQVGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRY
	orf39a	GQVAAPVIRLAQLWQDFQQVGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRY
40	orf39-1.pep	KADGRILQLDLNLRIRAGEVLGIVGRSGSGKSTLTKLVQRLYPVEQGRVLVDGNDLALAA
	orf39a	KADGRILQLDLNLRIRAGEVLGIVGRSGSGKSTLTKLVQRLYPVPAQGRVLVDGNDLALAA
45	orf39-1.pep	PAWLRRQVGVVLQENVLLNRSIRDNIALTDTGMPLERII EAAKLAGAHEFIMELPEGYGT
	orf39a	PAWLRRQVGVVLQENVLLNRSIRDNIALTDTGMPLERII EAAKLAGAHEFIMELPEGYGT
50	orf39-1.pep	VVGEQGAGLSGGQRQRIAIARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTV
	orf39a	VVGEQGAGLSGGQRQRIAIARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTV
55	orf39-1.pep	LIIAHLSTVKT AHRIIAMDKGRIVEAGTQQELLAKPNGYYRYLYDLQNGX
	orf39a	LIIAHLSTVKT AHRIIAMDKGRIVEAGTQQELLAKPNGYYRYLYDLQNGX

The complete length ORF39a nucleotide sequence <SEQ ID 29> is:

	1	ATGTCTATCG	TATCCGCACC	GCTCCCCGCC	CTTCCGCC	TCATCATCCT
55	51	CGCCCATAC	CACGGCATTG	CCGCCAATCC	TGCCGATATA	CAGCATGAAT
	101	TTTGTACTTC	CGCACAGAGC	GATTAAATG	AAACGCAATG	GCTGTTAGCC
	151	GCCAAATCTT	TGGGATTGAA	GGCAAAGGTA	GTCCGCCAGC	CTATTAAACG
	201	TTTGGCTATG	GCGACTTTAC	CCGCATTGGT	ATGGTGTGAT	GACGGCAACC
	251	ATTTTATTTT	GGCTAAAACA	GACGGTGGGG	GTGAGCATGC	CCAATATCTA
60	301	ATACAGGATT	TAACACGAA	TAAGTCTGCG	GTATTGTCTT	TTGCCGAATT
	351	TTCTAACAGA	TATTCGGGCA	AACTGATATT	GGTTGCTTCC	CGCGCTTCGG
	401	TATTGGGCAG	TTTGCCAAAG	TTTGACTTTA	CCTGGTTTAT	TCCGGCGGTA
	451	ATCAAATACC	GCCGGTTGTT	TTTGAAGTA	TTGGTGGTGT	CGGTGGTGT
	501	GCAGCTGTTT	GCGCTGATTA	CGCCTCTGTT	TTCCAAGTG	GTGATGGACA
	551	AGGTGCTGGT	ACATCGGGGA	TTCTCTACTT	TGGATGTGGT	GTCCGTGGCT
65	601	TTGTTGGTGG	TGTCGCTGTT	TGAGATTGTG	TTGGGCGGTT	TGCGGACGTA
	651	TCTGTTTGCA	CATACGACTT	CACGTATTGA	TGTGGAATTG	GGCGCGCGTT

5 701 TGTTCGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCTGA GCACAGACGA  
 751 GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATTT  
 801 CTTGACCGGT CAGGCGCTGA CTTCGGTGTG GGATTTGGCG TTTTCGTTTA  
 851 CTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA  
 901 TTGGCTTCGT TGCCTGCCTA TCGGTTTTGG TCGGCATTTA TCAGTCCGAT  
 951 ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCACT  
 1001 CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCATGGCG  
 1051 GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT  
 1101 GGCTTCGGGA TTTCCGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG  
 1151 TGCAGCTGAT TCAGAAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA  
 1201 CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA  
 1251 TATGCTCTCG GGACAGGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT  
 1301 GGCAGGATTT CCAGCAGGTG GGGATTTTCGG TGGCGCGTTT GGGGGAATTT  
 1351 CTGAATGCGC CGACCGAGAA TCGCTCTTCG CATTTGGCTT TGCCCGATAT  
 1401 CCGGGGGGAG ATTACGTTTC AACATGTCTGA TTTCCGCTAT AAGGCGGACG  
 1451 GCAGGCTGAT TTTGCAAGAT TTGAACCTGC GGATTCGGGC GGGGGAAGTG  
 1501 CTGGGGATTG TGGGACGTTT CCGGTCGGGC AAATCCACAC TCACCAATTT  
 1551 GGTGCAGCGT CTGTATGTAC CGGCGCAGGG ACGGTGTGTT GTGGACGGCA  
 1601 ACGATTTGGC TTTGGCCGCT CCTGCTTGGC TGGCGCGGCA GGTTCGGCGTG  
 1651 GTCTTGACAG AGAATGTGCT GCTCAACCGC AGCATAACCG ACAATATCGC  
 1701 GCTGACGGAT ACGGCTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC  
 1751 TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC  
 1801 GTGGTGGGCG AACAAGGGGC CGGCTTGTTC GGCAGGACAG GGCAGCGTAT  
 1851 TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTGTATG  
 1901 AAGCCACCGA CCGGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC  
 1951 ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT  
 2001 GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA  
 2051 TTGTGGAAGC GGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT  
 2101 TACCCTATC TGTATGATTT ACAGAACGGG TAG

30 This encodes a protein having amino acid sequence <SEQ ID 30>:

1 MSIVSAPLPA LSALIILAHY HGIAANPADI QHEFCTSAQS DLNETQWLLA  
 51 AKSLGLKAKV VRQPIKRLAM ATLPALVWCD DGNHFILAKT DGGGEHAQYL  
 101 IQDLTTNKSA VLSFAEFSNR YSGKLILVAS RASVLGSLAK FDFTWFIPAV  
 151 IKYRRLFFEV LVSVVLQLE ALITPLFFQV VMDKVLVHRG ESTLDVVSVA  
 201 LLVVSLEFIV LGGLRITYLFA HTTSRIDVEL GARLFRHLLS LPLSYFEHRR  
 251 VGDTVARVRE LEQIRNFLTG QALTSVLDLA FSFIFLAVMW YYSSTLTWV  
 301 LASLPAYAFW SAFISPIRLT RLNDKFARNA DNQSFLVESI TAVGTVKAMA  
 351 VEPQMTQRWD NQLAAYVASG FRVTKLAVVG QQGVQLIQKL VTVATLWIGA  
 401 RLVIKSLTV GQLIAFNMLS GQVAAPVIRL AQLWQDFQOV GISVARLGDI  
 451 LNAPTENASS HLALPDIRGE ITFEHVDFRY KADGRLLIQD LNLIRIRAGEV  
 501 LGIVGRSGSG KSTLTCLKVQR LYVPAQGRVL VDGNDLALAA PAWLRRQVGV  
 551 VLQENVLLNR SIRDNIALTD TGMPLERIE AAKLAGAHEF IMELPEGYGT  
 601 VVGEQGAGLS GGQRQRIATA RALITNPRIL IFDEATSALD YESERAIMQN  
 651 MQAICANRTV LIIAHLSTV KTAHRIIAMD KGRIVEAGTQ QELLAKPNGY  
 701 YRYLYDLQNG \*

ORF39a is homologous to a cytolysin from *A. pleuropneumoniae*:

sp|P26760|RT1B ACTPL RTX-I TOXIN DETERMINANT B (TOXIN RTX-I SECRETION ATP-BINDING PROTEIN) (APX-IB) (HLY-IB) (CYTOLYSIN IB) (CLY-IB)  
 >gi|97137|pir||D43599 cytolysin IB - Actinobacillus pleuropneumoniae (serotype 9)  
 >gi|38944 (X61112) ClyI-B protein [Actinobacillus pleuropneumoniae] Length = 707  
 Score = 931 bits (2379), Expect = 0.0  
 Identities = 472/690 (68%), Positives = 540/690 (77%), Gaps = 3/690 (0%)  
 Query: 20 YHGIAANPADIQHEFCTSAQSDLNETQWXXXXXXXXXXXXVVRQPIKRLAMATLPALVWC 79  
 YH IA NP +++H+F + L+ T W V++ I RLA LPALVW  
 Sbjct: 20 YHNIIVNPEELKHKFDLEGKG-LDLTAWLLAAKSLELKAKQVKKAIIDRLAFIALPALVWR 78  
 Query: 80 DDGNHFILAKTDGGGEHAQYLIQDLTTNKSAVLSFAEFSNRYSGKLILVASRASVLGSLA 139  
 +DG HFIL K D E +YLI DL T+ +L AEF + Y GKLILVASRAS++G LA  
 Sbjct: 79 EDGKHFILTKIDN--EAKKYLIFDLETHNPRILEQAEFESLYQGKLILVASRASIVGKLA 136  
 Query: 140 KFDFTWFIPAVIKYRXXXXXXXXXXXXXXXXXITPLFFQVMDKVLVHRGFXXXXXXXXXX 199  
 KFDFTWFIPAVIKYR+ ITPLFFQVMDKVLVHRGF  
 Sbjct: 137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVMDKVLVHRGFSTLNVITV 196  
 Query: 200 XXXXXXXFEIVLGGLRITYLFAHTTSRIDVELGARLFRHLLSLPLSYFEHRRVGDTVARVR 259

FEIVL GLRITY+FAH+TSRIDVELGARLFRHLL+LP+SYFE+RRVGD TVARVR  
 Sbjct: 197 ALAIVVLFEIVLNLRTYIFAHSTSRIDVELGARLFRHLLALPISYFENRRVGD TVARVR 256

5 Query: 260 ELEQIRNFLTGQALTSVLDLAFSFI FLAVMWYYSSTLTWVVLASLPAYAFWSAFISPILR 319  
 EL+QIRNFLTGQALTSVLDL FSFIF AVMWYYS LT V+L SLP Y WS FISPI LR  
 Sbjct: 257 ELDQIRNFLTGQALTSVLDL MFSFIFFAVMWYYS PKLTLVILGSLPFYMGWSIFISPILR 316

10 Query: 320 TRLNDKFARNADNQSF LVESITAVGTVKAMAVEPQMTQRWDNQLAAYVASGFRVTKLAVV 379  
 RL++KFAR ADNQSFLVES+TA+ T+KA+AV PQMT WD QLA+YV++GFRVT LA +  
 Sbjct: 317 RRLDEKFARGADNQSF LVESTAINTIKALAVTPQMTNTWDKQLASYVSAGFRVTTLATI 376

15 Query: 380 GQQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLSGQVAAPVIRLAQLWQDFQQ 439  
 GQQGVQ IQK+V V TLW+GA LVI L++GQLIAFNMLSGQV APVIRLAQLWQDFQQ  
 Sbjct: 377 GQQGVQFIQKVMVITLWLG AHLVISGDL SIGQLIAFNMLSGQVIAPVIRLAQLWQDFQQ 436

20 Query: 440 VGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRYKADGRLLIQDLNLRIRAGE 499  
 VGISV RLGD+LN+PTE+ LALP+I+G+ITF ++ FRYK D +IL D+NL I+ GE  
 Sbjct: 437 VGISVTRLGDVLNSPTESYQ GKALPEIKGDITFRNIRFRYKPDAPVILNDVNLSIQQGE 496

25 Query: 500 VLGIVGRSGSGKSTLT KL VQRLYVPAQGRVLVDGNDLALAAPAWLRRQVGVLQENVLLN 559  
 V+GIVGRSGSGKSTLT KL+QR Y+P G+VL+DG+DLALA P WLRQVGVLQ+NVLLN  
 Sbjct: 497 VIGIVGRSGSGKSTLT KL IQRFYIPENGQV LIDGHD LADPNWLRQVGVLQDNVLLN 556

30 Query: 560 RSIRDNIALTDTGMPLERIIEAAKLAGAHEFIMELPEGYGTVVGEQAGLSSGQRQRIAI 619  
 RSIRDNIAL D GMP+E+I+ AAKLAGAHEFI EL EGY T+VGEQAGLSSGQRQRIAI  
 Sbjct: 557 RSIRDNIALADPGMPMEKIVHAAKLAGAHEFISELREGYNTIVGEQAGLSSGQRQRIAI 616

35 Query: 620 ARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTVLIIAHLSTVKTAHRIIAM 679  
 ARAL+ NP+ILIFDEATSALDYESE IM+NM IC RTV+IIAHLSTVK A RII M  
 Sbjct: 617 ARALVNNPKILIFDEATSALDYESEHIIMRNMHQICKGRTVIIIAHLSTVKNADRIIVM 676

Query: 680 DKGRIVEAGTQQELLAKPNGYYRYLYDLQN 709  
 +KG+IVE G +ELLA PNG Y YL+ LQ+  
 Sbjct: 677 EKGQIVEQGKH KELLADPNGLYH LHLQLS 706

Homology with the HlyB leucotoxin secretion ATP-binding protein of *Haemophilus actinomycetemcomitans* (accession number X53955)

ORF39 and HlyB protein show 71% and 69% amino acid identity in 167 and 55 overlap at the N- and C-terminal regions, respectively:

40 Orf39 1 KFDFTWFIPAVIKYRXXXXXXXXXXXXXXXXXITPLFFQVMDKVLVHRGFXXXXXXXXXX 60  
 KFDFTWFIPAVIKYR+ ITPLFFQVMDKVLVHRGF  
 HlyB 137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVMDKVLVHRGFSTLNVITV 196

45 Orf39 61 XXXXXXFEIVLGLR TYLFAHTTSRIDVELGARLFRHLLSLPLSYFEHRRVGD TVARVR 120  
 FEI+LGGLR TY+FAH+TSRIDVELGARLFRHLL+LP+SYFE RRVGD TVARVR  
 HlyB 197 ALAIVVLFEIILGLR TYVFAHSTSRIDVELGARLFRHLLALPISYFEARRVGD TVARVR 256

50 Orf39 121 ELEQIRNFLTGQALTSVLDLAFSFI FLAVMWYYSSTLTWVVLASLIC 167  
 EL+QIRNFLTGQALTS+LDL FSFIF AVMWYYS LT VVL SL C  
 HlyB 257 ELDQIRNFLTGQALTSILDLLFSFIFFAVMWYYS PKLTLVVLGSLPC 303

//

55 Orf39 166 ICANRTVLIIAHLSTVKTAHRIIAMDKGRIVEAGTQQELLANXNGYYRYLYDLQ 220  
 IC NRTVLIIAHLSTVK A RII MDKG I+E G QELL + G Y YL+ LQ  
 HlyB 651 ICQNRTVLIIAHLSTVKNADRIIVMDKGEIIEQGKHQELLKDEKGLYSYHLQLQ 705

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

**Example 7**

60 The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 31>



```

1  ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT
51 CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA
101 ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT
151 GACGGGTTGA ACGCCCAAak sGACGCCGAA ATCAGA...

```

5 This corresponds to the amino acid sequence <SEQ ID 32; ORF52>:

```

1  MKYLLRTALL AVAAAGIYAC QPQSEAAVQV KAENSLTAMR LAVADKQAEI
51 DGLNAQXDAE IR..

```

Further work revealed the complete nucleotide sequence <SEQ ID 33>:

```

10      1  ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT
        51 CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA
        101 ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT
        151 GACGGGTTGA ACGCCCAAAT CGACGCCGAA ATCAGACAAC GCGAAGCCGA
        201 AGAATTGAAA GACTACCGAT GGATACACGG CGACGCCGAA GTGCCGGAGC
        251 TGGAAAAATG A

```

15 This corresponds to the amino acid sequence <SEQ ID 34; ORF52-1>:

```

1  MKYLLRTALL AVAAAGIYAC QPQSEAAVQV KAENSLTAMR LAVADKQAEI
51 DGLNAQIDAE IRQREAEELK DYRWIHGDAE VPELEK*

```

Computer analysis of this amino acid sequence predicts a prokaryotic membrane lipoprotein lipid attachment site (underlined).

20 ORF52-1 (7kDa) was cloned in the pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 4A shows the results of affinity purification of the GST-fusion. Figure 4B shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF52-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could  
 25 be useful antigens for vaccines or diagnostics.

### Example 8

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 35>

```

30      1  ATGGTTATCG GAATATTACT CGCATCAAGC AAGCATGCTC TTGTCATTAC
        51 TCTATTGTTA AATCCCGTCT TCCATGCATC CAGTTGCGTA TCGCGTTsGG
        101 CAATACGGAA TAAAAtCTGC TGTTCTGCTT TGGCTAAATT TGCCAAATTG
        151 TTTATTGTTT CTTTAGGAGC AGCTTGCTTA GCCGCCTTCG CTTTCGACAA
        201 CGCCCCACA GGCGCTTCCC AAGCgTTGCC TACCGTTACC GCACCCGTGG
        251 CGATTCCCGC GCCCGCTTCG GCAGCCTGA

```

This corresponds to the amino acid sequence <SEQ ID 36; ORF56>:

```

35      1  MVIGILLASS KHALVITLLL NPVFHASSCV SRXAIRNKIC CSALAKFAKL
        51 FIVSLGAACL AAFAFDNAPT GASQALPTVT APVAIPAPAS AA*

```

Further work revealed the complete nucleotide sequence <SEQ ID 37>:

```

1  ATGGCTTGTA CAGGTTTGAT GGT'TTTTCCG TTAATGGTTA TCGGAATATT

```

5

```

51  ACTTGCATCA AGCAAGCCTG CTCCTTTCCT TACTCTATTG TTAAATCCCG
101 TCTTCCATGC ATCCAGTTGC GTATCGCGTT GGGCAATACG GAATAAAATC
151 TGCTGTTCTG CTTTGGCTAA ATTTGCCAAA TTGTTTATTG TTTCTTTAGG
201 AGCAGCTTGC TTAGCCGCCT TCGCTTTCGA CAACGCCCCC ACAGGCGCTT
251 CCAAGCGTT GCCTACCGTT ACCGCACCCG TGGCGATTCC CGCGCCCGCT
301 TCGGCAGCCT GA

```

This corresponds to the amino acid sequence <SEQ ID 38; ORF56-1>:

10

```

1  MACTGLMVFP LMVIGILLAS SKPAPFLTLL LNPVFHASSC VSRWAIRNKI
51  CCSALAKFAK LFIVSLGAAC LAAFAFDNAP TGASQALPTV TAPVAIPAPA
101 SAA*

```

Computer analysis of this amino acid sequence predicts a leader peptide (underlined) and suggests that ORF56 might be a membrane or periplasmic protein.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

## 15 Example 9

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 39>

20

```

1  ATGTTCACTA TTTTAAATGT GTTCTTTCAT TGTATTCTGG CTTGTGTAGT
51  CTCTGGTGAG ACGCCTACTA TATTTGGTAT CCTTGCTCTT TTTTACTTAT
101 TGTATCTTTC TTATCTTGCT GTTTTAAAGA TTTTCTTTTC TTTTCTTCTA
151 GACAGAGTTT CACTCCGGTC TCCCAGGCTG GAGTGCAAAAT GGCATGACCC
201 TTTGGCTCAC TGGCTCACGG CCACTTCTGC TATTCTGCCG CCTCAGCCTC
251 CAGGG...

```

This corresponds to the amino acid sequence <SEQ ID 40; ORF63>:

25

```

1  MFSILNVFLH CILACVVSSE TPTIFGILAL FYLLYLSYLA VFKIFFSFFL
51  DRVSLRSPRL ECKWHDFLAH WLTATSAILP PQPPG...

```

Computer analysis of this amino acid sequence predicts a transmembrane region.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

## Example 10

30 The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 41>

35

```

1  ..GTGCGGACGT GGTGGTTTTT TTGGTTGCAG CGTTTGAAAT ACCCGTTGTT
51  GCTTTGGATT GCGGATATGT TGCTGTACCG GTTGTGGGC GCGCGGAAA
101 TCGAATGCGG CCGTTGCCCT GTGCCGCCGA TGACGGATTG GCAGCATTTT
151 TTGCCGCCGA TGGGAACGGT GTCGGCTTGG GTGGCGGTGA TTTGGGCATA
201 CCTGATGATT GAAAGTGAAA AAAACGGAAG ATATTGA

```

This corresponds to the amino acid sequence <SEQ ID 42; ORF69>:

```

1  ..VRTWLVFVLQ RLKYPALLWI ADMLLYRLLG GAEIECGRCP VPPMTDWQHF
51  LPAMGTVSAW VAVIWAYLMI ESEKNGRY*

```

Computer analysis of this amino acid sequence predicts a transmembrane region.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

Homology with a predicted ORF from *N.meningitidis* (strain A)

ORF69 shows 96.2% identity over a 78aa overlap with an ORF (ORF69a) from strain A of *N. meningitidis*:

```

      10      20      30      40      50      60
orf69.pep  VRTWL VFWLQRLKYPLLLWIADMLLYRLLGGAEIECGRCVPVPPMTDWQHFLPAMGTVSAW
            |||||
orf69a     VRTWL VFWLQRLKYPLLLCIADMLLYRLLGGAEIECGRCVPVPPMTDWQHFLPTMGTVAAW
            10      20      30      40      50      60

      70      79
orf69.pep  VAVIWAYLMIESEKNGRYX
            |||||
orf69a     VAVIWAYLMIESEKNGRYX
            70

```

The ORF69a nucleotide sequence <SEQ ID 43> is:

```

      1  GTGCGGACGT  GGTGGTTTTT  TTGGTTGCAG  CGTTTGAAAT  ACCCGTTGTT
     51  GCTTTGTATT  GCGGATATGC  TGCTGTACCG  GTTGTGGGGC  GGCGCGGAAA
    101  TCGAATGCGG  CCGTTGCCCT  GTACCGCCGA  TGACGGATTG  GCAGCATTTT
    151  TTGCCGACGA  TGGGAACGGT  GGC GGCTTG  GTGGCGGTGA  TTTGGGCATA
    201  CCTGATGATT  GAAAGTGAAA  AAAACGGAAG  ATATTGA

```

This encodes a protein having amino acid sequence <SEQ ID 44>:

```

      1  VRTWL VFWLQ  RLKYPLLLCI  ADMLLYRLLG  GAEIECGRCP  VPPMTDWQHF
     51  LPTMGTVAAW  VAVIWAYLMI  ESEKNGRY*

```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

**Example 11**

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 45>

```

      1  ATGTTTCAAA  ATTTTGATTT  GGGCGTGTTT  CTGCTTGCCG  TCCTCCCCGT
     51  GCTGCCCTCC  ATTACCGTCT  CGCACGTGGC  GCGCGGCTAT  ACGGCGCGCT
    101  ACTGGGGAGA  CAACACTGCC  GAACAATACG  GCAGGCTGAC  ACTGAACCCC
    151  CTGCCCCATA  TCGATTGGT  CGGCACAATC  ATCgTACCGC  TGCTTACTTT
    201  GATGTTACAG  CCCTTCCTGT  TCGGCTGGGC  GCGTCCGATT  CCTATCGATT
    251  CGCGCAACTT  CCGCAACCCG  cGCCTTGCC  GCGGTTGCGT  TGCCGCGTCC
    301  GGCCCGCTGT  CGAATCTAGC  GATGGCTGTW  CTGTGGGGCG  TGGTTTTGGT
    351  GCTGACTCCG  TATGTCGGCG  GGGCGTATCA  GATGCCGTTG  GCTCAAATGG
    401  CAAACTACGG  TATTCTGATC  AATGCGATT  TGTTCCGCGT  CAACATCATC
    451  CCCATCCTGC  CTTGGGACGG  CGGCATTTTC  ATCGACACCT  TCCTGTCGGC
    501  GAAATATTCC  CAAGCGTTCC  GCAAAATCGA  ACCTTATGGG  ACGTGGATTA
    551  TCCTACTGCT  GATGCTGACC  sGGGTTTGG  GTGCGTTTAT  wGCACCGATT
    601  sTGCGGmTGc  GTGATTGCrT  TTGTGCAGAT  GTwCGTCTGA  CTGGCTTTCA
    651  GACGGCATAA

```

This corresponds to the amino acid sequence <SEQ ID 46; ORF77>:

1 MFQNF~~DLGVF~~ LLAVLPVLPS ITVSHVARGY TARYWGDNTA EQYGR~~LT~~LN~~P~~  
 51 LPHIDLVGTI IVPL~~LT~~LMFT PFLFGWARPI PIDSRNFRNP RLAWRCVAAS  
 101 GPLSNLAMAV LWGVVLVLT~~P~~ YVGGAYQMPL AQMANYGILI NAILFALNII  
 151 PILPWDGGIF IDTFLSAKYS QAFRKIEPYG TWIILLMLT XVLGA~~FI~~API  
 201 XR~~RD~~CXCAD VRLTGFQTA\*

Further work revealed the complete nucleotide sequence <SEQ ID 47>:

1 ATGTTTCAAA ATTTTGATTT GGGCGTGTTT CTGCTTGCCG TCCTGCCCCG  
 51 GCTGCTCTCC ATTACCGTCA GGGAGGTGGC GCGCGGCTAT ACGGCGCGCT  
 101 ACTGGGGAGA CAACACTGCC GAACAATACG GCAGGCTGAC ACTGAACCCC  
 151 CTGCCCCATA TCGATTGGT CGGCACAATC ATCGTACCGC TGCTTACTTT  
 201 GATGTTACAG CCTTCCTGT TCGGCTGGGC GCGTCCGATT CCTATCGATT  
 251 CGCGCAACTT CCGCAACCCG CGCCTTGCTT GCGTTCGCTT TGCCGCGTCC  
 301 GGCCCGCTGT CGAATCTAGC GATGGCTGTT CTGTGGGGCG TGGTTTTGGT  
 351 GCTGACTCCG TATGTCGGCG GGGCGTATCA GATGCCGTTG GCTCAAATGG  
 401 CAACTACGG TATTCTGATC AATGCGATTC TGTTCGCGCT CAACATCATC  
 451 CCCATCCTGC CTGGGACGG CGGCATTTTC ATCGACACCT TCCTGTCCGGC  
 501 GAAATATTCT CAAGCGTTC CAAAAATCGA ACCTTATGGG ACGTGGATTA  
 551 TCCTACTGCT GATGCTGACC GGGGTTTTGG GTGCGTTTAT TGCACCGATT  
 601 GTGCGGCTGG TGATTGCGTT TGTGCAGATG TTCGTCTGA

20 This corresponds to the amino acid sequence <SEQ ID 48; ORF77-1>:

1 MFQNF~~DLGVF~~ LLAVLPVLLS ITVREVARGY TARYWGDNTA EQYGR~~LT~~LN~~P~~  
 51 LPHIDLVGTI IVPL~~LT~~LMFT PFLFGWARPI PIDSRNFRNP RLAWRCVAAS  
 101 GPLSNLAMAV LWGVVLVLT~~P~~ YVGGAYQMPL AQMANYGILI NAILFALNII  
 151 PILPWDGGIF IDTFLSAKYS QAFRKIEPYG TWIILLMLT GVLGA~~FI~~API  
 201 VRLVIAFVQM FV\*

Computer analysis of this amino acid sequence reveals a putative leader sequence and several transmembrane domains.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

#### Homology with a predicted ORF from *N.meningitidis* (strain A)

30 ORF77 shows 96.5% identity over a 173aa overlap with an ORF (ORF77a) from strain A of *N.meningitidis*:

		10	20	30	40	50	60
orf77.pep		MFQNF <del>DLGVF</del> LLAVLPVLPSITVSHVARGYTARYWGDNTAEQYGR <del>LT</del> LNPLPHIDLVGTI					
35	orf77a			RGYTARYWGDNTAEQYGR <del>LT</del> LNPLPHIDLVGTI			
				10	20	30	
		70	80	90	100	110	120
40	orf77.pep	IVPL <del>LT</del> LMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLT <del>P</del>					
	orf77a	IVPL <del>LT</del> LMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLT <del>P</del>					
		40	50	60	70	80	90
		130	140	150	160	170	180
45	orf77.pep	YVGGAYQMPLAQMANYGILINAILFALNIIPILPWDGGIFIDTFLSAKYSQAFRKIEPYG					
	orf77a	YVGGAYQMPLAQMANYXILINAILXALNIIPILPWDGGIFIDTFLSAKXSQAFRKIEPYG					
		100	110	120	130	140	150
50		190	200	210	220		
	orf77.pep	TWIILLMLTXVLGA <del>FI</del> APIXR <del>RD</del> CXCADVRLTGFQTA <del>X</del>					
	orf77a	TWII <del>X</del> LLMLTGVLGAXIAPIVQLVIAFVQMFVX					
		160	170	180			

ORF77-1 and ORF77a show 96.8% identity in 185 aa overlap:

```

      10      20      30      40      50      60
orf77-1.pep MFQNFDLGVFLLA VLPVLLSITVRE VARGYTARYWGDNTAEQYGR LTLNPLPHIDL VGTI
5 orf77a      |||||
      10      20      30
      70      80      90      100     110     120
orf77-1.pep IVPLLTLMFT PFLFGWARPIPIDSRNFRNPRLAWRCVAASG PLSNLA MAVLWGVVLVLT P
10 orf77a      IVPLLTLMFT PFLFGWARPIPIDSRNFRNPRLAWRCVAASG PLSNLA MAVLWGVVLVLT P
      40      50      60      70      80      90
      130     140     150     160     170     180
orf77-1.pep YVGGAYQMPLAQMANYGILINAILFALNIIPILPWDGGIFIDTFLSAKYSAFRKIEPYG
15 orf77a      YVGGAYQMPLAQMANYXILINAILXALNIIPILPWDGGIFIDTFLSAKXSAFRKIEPYG
      100     110     120     130     140     150
      190     200     210
orf77-1.pep TWIILLMLTGV LGAFIAPIVRLVIAFVQMFVX
20 orf77a      TWIIXLLMLTGV LGAXIAPIVQLVIAFVQMFVX
      160     170     180

```

A partial ORF77a nucleotide sequence <SEQ ID 49> was identified:

```

      1  ..CGCGGCTATA CAGCGCGCTA CTGGGGTGAC AACACTGCCG AACAAATACGG
      51 CAGGCTGACA CTGAACCCCC TGCCCCATAT CGATTGGTC GGCACAATCA
30 101 TCGTACCGCT GCTTACTTTG ATGTTTACGC CCTTCCTGTT CGGCTGGGCG
      151 CGTCCGATTC CTATCGATTC GCGCAACTTC CGCAACCCGC GCCCTGCCTG
      201 GCGTTGCGTT GCCGCGTCCG GCCCGCTGTC GAATCTGGCG ATGGCTGTTC
      251 TGTGGGGCGT GGTTTTGGTG CTGACTCCGT ATGTCGGTGG GGCGTATCAG
      301 ATGCCGTTGG CNCAAATGGC AAACACNNN ATTCTGATCA ATGCGATTCT
35 351 GTNCGCGCTC AACATCATCC CCATCCTGCC TTGGGACGGC GGCATTTTCA
      401 TCGACACCTT CCTGTCCGCN AAATANTCGC AAGCGTCCG CAAATCGAA
      451 CCTTATGGGA CGTGGATTAT CCNGCTGCTT ATGCTGACCG GGGTTTGGG
      501 TCGGTNTATT GCACCGATTG TGCAGCTGGT GATTGCGTTT GTGCAGATGT
      551 TCGTCTGA

```

This encodes a protein having amino acid sequence <SEQ ID 50>:

```

40      1  ..RGYTARYWGD NTAEQYGR LTLNPLPHIDL VGTIIVPLLT LMFT PFLFGWA
      51 RPIPIDSRNF RNPRLAWRCV AASG PLSNLA MAVLWGVVLV LTPYVGGAYQ
      101 MPLAQMANYX ILINAILXAL NIIPILPWDG GIFIDTFLSA KXSQAFRKIE
      151 PYGTWIIIXLL MLTGV LGAXI APIVQLVIAF VQMFV*

```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could  
 45 be useful antigens for vaccines or diagnostics.

### Example 12

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 51>

```

      1  ATGAACCTGA TTTACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT
      51 TTACGCGCTC CTTGCCTTCC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT
50 101 ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAATGCTG
      151 GGCTACACCG CCTCAAAAT GCCGCGCGC GCCTACGAAC TGATCCCCCT
      201 CGCCGTCCTT ATCGGCGGAC TGGTCTCCCT CAGCCAGCTT GCCGCGGCA
      251 GCGAACTGAC CGTCATCAA GGCAGCGGCA TGAGACCAA AAAGCTGCTG
      301 TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT
55 351 CGGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG

```

401 CCGCCGCCAT CAACGGCAAA ATCAGCACCG GCAATACCGG CCTTTGGCTG  
 451 AAAGAAAAAA ACAGCGTGAT CAATGTGCGC GAAATGTTGC CCGACCAT..

This corresponds to the amino acid sequence <SEQ ID 52; ORF112>:

5 1 MNLISRYIIR QMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEML  
 51 GYTALKMPAR AYELIPLAVL IGGLVLSLSQL AAGSELTVIK ASGMSTKKLL  
 101 LILSQFGFIF AIATVALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL  
 151 KEKNSVINVR EMLPDH...

Further work revealed further partial nucleotide sequence <SEQ ID 53>:

10 1 ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT  
 51 TTACGCGCTC CTTGCCCTTC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT  
 101 ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAAATGCTG  
 151 gGGTACACCG CCTCAAAAT GCCCGCCCGC GCCTACGAAC TGATTCCCCT  
 201 CGCCGTCCTT ATCGGCGGAC TGGTCTCCCT CAGCCAGCTT GCCGCGGGCA  
 15 251 GCGAACTGAC CGTCATCAAA GCCAGCGGCA TGAGCACCAA AAAGCTGCTG  
 301 TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT  
 351 CCGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG  
 401 CCGCCGCCAT CAACGGCAAA ATCAGCACCG GCAATACCGG CCTTTGGCTG  
 451 AAAGAAAAAA ACAGC rTkAT CAATGTGCGC GAAATGTTGC CCGACCATAC  
 501 GCTTTTGGGC ATCAAAATTT GGGCGCGCAA CGATAAAAAC GAATTGGCAG  
 20 551 AGGCAGTGGA AGCCGATTCC GCCGTTTGA ACAGCGACGG CAGTTGGCAG  
 601 TTGAAAAACA TCCGCCGAG CACGCTTGGC GAAGACAAAG TCGAGGTCTC  
 651 TATTGCGGCT GAAGAAAAC TGGCGATTTC CGTCAAACGC AACCTGATGG  
 701 ACGTATTGCT CGTCAAACCC GACCAAATGT CCGTCGGCGA ACTGACCACC  
 25 751 TACATCCGCC ACCTCCAAAA CAACAGCAA AACACCCGAA TCTACGCCAT  
 801 CGCATGGTGG CGCAAATTGG TTTACCCCGC CGCAGCCTGG GTGATGGCGC  
 851 TCGTCGCCTT TGCCTTTACC CCGCAAACCA CCGCCACGG CAATATGGGC  
 901 TTAATACTCT TCGGCGGCAT CTGTsTCGGA TTGCTGTTC ACCTTGCCGG  
 951 ACGGCTCTTT GGGTTTACCA GCCAACTCGG...

This corresponds to the amino acid sequence <SEQ ID 54; ORF112-1>:

30 1 MNLISRYIIR QMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEML  
 51 GYTALKMPAR AYELIPLAVL IGGLVLSLSQL AAGSELTVIK ASGMSTKKLL  
 101 LILSQFGFIF AIATVALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL  
 151 KEKNSXINVR EMLPDHTLLG IKIWARNDKN ELAEAVEADS AVLNSDSGWQ  
 201 LKNIRRSTLG EDKVEVSIAA EENWPISVKR NLMDVLLVKP DQMSVGELTT  
 35 251 YIRHLQNSQ NTRIYAIWW RKLVPAAAW VMALVAFAT PQTTRHGNMG  
 301 LKLFGGICXG LLFHLAGRLF GFTSQL...

Computer analysis of this amino acid sequence predicts two transmembrane domains.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

#### Homology with a predicted ORF from *N.meningitidis* (strain A)

40 ORF112 shows 96.4% identity over a 166aa overlap with an ORF (ORF112a) from strain A of *N.meningitidis*:

		10	20	30	40	50	60
45	orf112.pep	MNLISRYIIRQMAVMAVYALLAFLALYSFF	EILYETGNLGKGSYGIWEMLGYTALKMPAR				
	orf112a	MNLISRYIIRQMAVMAVYALLAFLALYSFF	EILYETGNLGKGSYGIWEMXGYTALKMXAR				
		10	20	30	40	50	60
50	orf112.pep	AYELIPLAVLIGGLVLSLSQLAAGSELTVIKASGMSTKKLL	LILSQFGFIFAIATVALGEW				
	orf112a	AYELMPLAVLIGGLVXSQLAAGSELXVIKASGMSTKKLL	LILSQFGFIFAIATVALGEW				
		70	80	90	100	110	120

5

	130	140	150	160	
orf112.pep	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSVINVREMLPDH				
orf112a	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSIINVREMLPDHTLLGIKIWARNDKN				
	130	140	150	160	170 180
orf112a	ELAEAVEADSAVLNSDGSWQLKNIRRLSTLGEDKVEVSIAAEEXWPISVKNRLMDVLLVKP				
	190	200	210	220	230 240

A partial ORF112a nucleotide sequence <SEQ ID 55> was identified:

10

1	ATGAACCTGA	TTTCACGTTA	CATCATCCGT	CAAATGGCGG	TTATGGCGGT
51	TTACGCGCTC	CTTGCCCTTC	TCGCTTTGTA	CAGCTTTTTT	GAAATCCTGT
101	ACGAAACCGG	CAACCTCGGC	AAAGGCAGTT	ACGGCATATG	GGAAATGNTG
151	GGNTACACCG	CCCTCAAAAT	GNCCGCCCGC	GCCTACGAAC	TGATGCCCTT
201	CGCCGTCCTT	ATCGGCGGAC	TGGTCTCTNT	CAGCCAGCTT	GCCGCCGGCA
15	251	GCGAACTGAN	CGTCATCAAA	GCCAGCGGCA	TGAGCACCAA
301	TTGATTCTGT	CGCAGTTCGG	TTTTATTTTT	GCTATTGCCA	CCGTCGCGCT
351	CGGCGAATGG	GTTGCGCCCA	CACTGAGCCA	AAAAGCCGAA	AACATCAAAG
401	CCGCGGCCAT	CAACGGCAAA	ATCAGTACCG	GCAATACCGG	CCTTTGGCTG
451	AAAGAAAAAA	ACAGCATTAT	CAATGTGCGC	GAAATGTTGC	CCGACCATAC
20	501	CCTGCTGGGC	ATTAAAATCT	GGGCCCGCAA	CGATAAAAAC
551	AGGCAGTGGA	AGCCGATTCC	GCCGTTTTGA	ACAGCGACGG	CAGTTGGCAG
601	TTGAAAAACA	TCCGCCGCAG	CACGCTTGGC	GAAGACAAAG	TCGAGGTCTC
651	TATTGCGGCT	GAAGAAAANT	GGCCGATTTC	CGTCAAACGC	AACCTGATGG
25	701	ACGTATTGCT	CGTCAAACCC	GACCAAATGT	CCGTCGGCGA
751	TACATCCGCC	ACCTCCAAAN	NNACAGCCAA	AACACCCGAA	TCTACGCCAT
801	CGCATGGTGG	CGCAAATTGG	TTTACCCCGC	CGCAGCCTGG	GTGATGGCGC
851	TCGTCGCTT	TGCCTTTACC	CCGCAAACCA	CCCGCCACGG	CAATATGGGC
901	TTAAAANTCT	TCGGCGGCAT	CTGTCTCGGA	TTGCTGTTCC	ACCTTGCCCG
951	NCGGCTCTTC	NGGTTTACCA	GCCAACTCTA	CGGCATCCCG	CCCTTCCTCG
30	1001	NCGGCGCACT	ACCTACCATA	GCCTTCGCCT	TGCTCGCCGT
1051	CGCAAACAGG	AAAAACGCTA	A		

This encodes a protein having amino acid sequence <SEQ ID 56>:

35

1	MNLISRYIIR	QMAMVAVYAL	LAFLALYSFF	EILYETGNLG	KGSYGIWEMX
51	GYTALKMXAR	AYELMPLAVL	IGGLVSXSQ	AAGSELXVIK	ASGMSTKKLL
101	LILSQFGFIF	AIATVALGEW	VAPTLSQKAE	NIKAAAINGK	ISTGNTGLWL
151	KEKNSIINVR	EMLPDHTLLG	IKIWARNDKN	ELAEAVEADS	AVLNSDGSWQ
201	LKNIRRLSTL	EDKVEVSIAA	EEXWPISVKN	NLMDVLLVKP	DQMSVGELTT
251	YIRHLQXXSQ	NTRIYAIWW	RKLVPAAAW	VMALVAFAPT	PQTRHGNMG
301	LKXFGGICLG	LLFHLAGRLE	XFTSQLYGIP	PFLXGALPTI	AFALLAVWLI
351	RKQEK*				

ORF112a and ORF112-1 show 96.3% identity in 326 aa overlap:

45

orf112a.pep	MNLISRYIIRQMAMVAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMXGYTALKMXAR
orf112-1	MNLISRYIIRQMAMVAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMLGYTALKMPAR
orf112a.pep	AYELMPLAVLIGGLVSXSQLAAGSELXVIKASGMSTKKLLLILSQFGFIFAIATVALGEW
orf112-1	AYELIPLAVLIGGLVLSQLAAGSELTVIKASGMSTKKLLLILSQFGFIFAIATVALGEW
orf112a.pep	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSIINVREMLPDHTLLGIKIWARNDKN
orf112-1	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSXINVREMLPDHTLLGIKIWARNDKN
orf112a.pep	ELAEAVEADSAVLNSDGSWQLKNIRRLSTLGEDKVEVSIAAEEXWPISVKNRLMDVLLVKP
orf112-1	ELAEAVEADSAVLNSDGSWQLKNIRRLSTLGEDKVEVSIAAEENWPISVKNRLMDVLLVKP
orf112a.pep	DQMSVGELTTYIRHLQXXSQNTRIYAIWWRKLVYPAAAWVMALVAFAPTQPTRHGNMG
orf112-1	DQMSVGELTTYIRHLQNNSQNTRIYAIWWRKLVYPAAAWVMALVAFAPTQPTRHGNMG
orf112a.pep	LKXFGGICLGLLFLHLAGRLFXFTSQLYGIPPFLXGALPTIAFALLAVWLIRKQEKRX

orf112-1

LKLFGGICXGLLFHLAGRLFGFTSQL

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

### 5 Example 13

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 57>

```

1   ..GCAGTAGCCG AAAC TGCCAA CAGCCAGGGC AAAGGTAAAC AGGCAGGCAG
51  TTCGGTTTCT GTTTCAC TGA AAAC TTCAGG CGACCTTTGC GGCAAAC TCA
101 AAACCACCTT TAAAACTTTG GTCTGCTCTT TGGTTTCCCT GAGTATGGTA
151 TTGCGTGCCC ATGCCCAAAT TACCACCGAC AAATCAGCAC CTAAAAACCA
201 GCAGGTCGTT ATCCTTAAAA CCAACACTGG TGCCCCCTTG GTGAATATCC
251 AAAC TCCGAA TGGACGCGGA TTGAGCCACA ACCGCTA.TA CGCATTTGAT
301 GTTGACAACA AAGGGGCAGT GTTAAACAAC GACCGTAACA ATAATCCGTT
351 TGTGGTCAAA GGCAGTGCGC AATTGATTTT GAACGAGGTA CGCGGTACGG
15  CTAGCAAACT CAACGGCATC GTTACCGTAG GCGGTCAAAA GGCCGACGTG
401 ATTATTGCCA ACCCCAACGG CATTACCGTT AATGGCGGCG GCTTTAAAAA
451 TGTCCGTCGG GGCATCTTAA CTACCGGTGC GCCCAAATC GGCAAAGACG
501 GTGCACTGAC AGGATTTGAT GTGCGTCAAG GCACATTGgA CCGTAGrAGC
551 AGCAGGTTGG AATGATAAAG GCGGAGCmrm yTACACCGGG GTACTTGCTC
601 GTGCAGTTGC TTTGCAGGGG AAATTwmMG GTAAA.AACT GGCGGTTTCT
20 701 ACCGGTCCTC AGAAAGTAGA TTACGCCAGC GCGGAAATCA GTGCAGGTAC
751 GGCAGCGGGT ACGAAACCGA CTATTGCCCT TGATACTGCC GCACTGGGCG
801 GTATGTACGC CGACAGCATC AACTGATTG CCAATGAAAA AGGCGTAGGC
851 GTCTAA

```

25 This corresponds to the amino acid sequence <SEQ ID 58; ORF114>:

```

1   ..AVAETANSQG KGKQAGSSVS VSLKTSGLDL GKLKTTLKLTL VCSLVSLSMV
51  LPAHAQITTD KSAPKNQQVV ILKTN TGAPL VNIQTFN GRG LSHNRXYAFD
101 VDNKGAVLNN DRNNNPFVVK GSAQLILNEV RGTASKLNGI VTVGGQKADV
151 IIANPNGITV NGGGFKNVGR GILTTGAPQI GKDGALTGFD VVKAHWTVXA
30 201 AGWNDKGGAX YTGVLARAVA LQKXXGKXL AVSTGPQKVD YASGEISAGT
251 AAGTKPTIAL DTAALGGMYA DSITLIANEK GVG*

```

Further work revealed the complete nucleotide sequence <SEQ ID 59>:

```

1   ATGAATAAAG GTTTACATCG CATTATCTTT AGTAAAAAGC ACAGCACCAT
35 51  GGTTCAGTA GCCGAAACTG CCAACAGCCA GGGCAAAGGT AAACAGGCAG
101 GCAGTTCGGT TTCTGTTTCA CTGAAAAC TT CAGGCGACCT TTGCGGCAAA
151 CTCAAAACCA CCCTTAAAC TTTGGTCTGC TCTTTGGTTT CCCTGAGTAT
201 GGTATTGCCT GCCATGCCC AAATTACCAC CGACAAATCA GCACCTAAAA
251 ACCAGCAGGT CGTTATCCTT AAAACCAACA CTGGTGCCCC CTTGGTGAAT
301 ATCCAAACTC CGAATGGACG CGGATTGAGC CACAACCGCT ATACGCAGTT
40 351 TGATGTTGAC AACAAAGGGG CAGTGTTAAA CAACGACCGT AACAAATAATC
401 CGTTTGTGGT CAAAGGCAGT GCGCAATTGA TTTTGAACGA GGTACGCGGT
451 ACGGCTAGCA AACTCAACGG CATCGTTACC GTAGGCGGTC AAAAGGCCGA
501 CGTGATTATT GCCAACCCCA ACGGCATTAC CGTTAATGGC GGCGGCTTTA
551 AAAATGTCGG TCGGGGCATC TTAAC TACCG GTGCGCCCCA AATCGGCAAA
45 601 GACGGTGCAT TGACAGGATT TGATGTGCGT CAAGGCACAT TGACCGTAGG
651 AGCAGCAGGT TGAATGATA AAGGCGGAGC CGACTACACC GGGGTACTTG
701 CTCGTGCAGT TGCTTTGCAG GGGAAATTAC AGGGTAAAAA CCTGGCGGTT
751 TCTACCGGTC CTCAGAAAGT AGATTACGCC AGCGGCGAAA TCAGTGCAGG
801 TACGGCAGCG GGTACGAAAC CGACTATTGC CCTTGATACT GCCGCACTGG
50 851 GCGGTATGTA CGCCGACAGC ATCACACTGA TTGCCAATGA AAAAGGCGTA
901 GGCCTCAAAA ATGCCGGCAC ACTCGAAGCG GCCAAGCAAT TGATTGTGAC
951 TTCGT CAGGC CGCATTGAAA ACAGCGGCCG CATCGCCACC ACTGCCGACG
1001 GCACGAAGC TTCACCGACT TATCTCTCCA TCGAAACCAC CGAAAAGGA
1051 GCGGCAGGCA CATTATTCTC CAATGGTGGT CGGATCGAGA GCAAAGGCTT
55 1101 ATTGGTTATT GAGACGGGAG AAGATATCAG CTTGCGTAAC GGAGCCGTGG
1151 TGCAGAATAA CGGCAGTCGC CCAGCTACCA CGGTATTAAA TGCTGGTCAT
1201 AATTTGGTGA TTGAGAGCAA AACTAATGTG AACAATGCCA AAGGCCCGGC

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1251	TACTCTGTCTG	GCCGACGGCC	GTACCGTCAT	CAAGGAGGCC	AGTATTCAGA
1301	CTGGCACTAC	CGTATACAGT	TCCAGCAAAG	GCAACGCCGA	ATTAGGCAAT
1351	AACACACGCA	TTACCGGGGC	AGATGTTACC	GTATTATCCA	ACGGCACCAT
1401	CAGCAGTTCC	GCCGTAATAG	ATGCCAAAGA	CACCGCACAC	ATCGAAGCAG
1451	GCAAACCGCT	TTCTTTGGAA	GCTTCAACAG	TTACCTCCGA	TATCCGCTTA
1501	AACGGAGGCA	GTATCAAGGG	CGGCAAGCAG	CTTGCTTTAC	TGGCAGACGA
1551	TAACATTACT	GCCAAAACCTA	CCAATCTGAA	TACTCCCGGC	AATCTGTATG
1601	TTCATACAGG	TAAAGATCTG	AATTTGAATG	TTGATAAAGA	TTTGTCTGCC
1651	GCCAGCATCC	ATTTGAAATC	GGATAACGCT	GCCCATATTA	CCGGCACCAG
1701	TAAAACCCCTC	ACTGCCTCAA	AAGACATGGG	TGTGGAGGCA	GGCTCGCTGA
1751	ATGTTACCAA	TACCAATCTG	CGTACCAACT	CGGGTAATCT	GCACATTCTG
1801	GCAGCCAAAG	GCAATATTCA	GCTTCGCAAT	ACCAAGCTGA	ACGCAGCCAA
1851	GGCTCTCGAA	ACCACCGCAT	TGCAGGGCAA	TATCGTTTCA	GACGGCCTTC
1901	ATGCTGTTTC	TGCAGACGGT	CATGTATCCT	TATGGGCCAA	CGGTAATGCC
1951	GACTTTTACCG	GTCACAATAC	CCTGACAGCC	AAGGCCGATG	TCAATGCAGG
2001	ATCGGTTGGT	AAAGGCCGTC	TGAAAGCAGA	CAATACCAAT	ATCACTTCAT
2051	CTTCAGGAGA	TATTACGTTG	GTTGCCGGCA	ACGGTATTCA	GCTTGGTGAC
2101	GGAAAACAAC	GCAATTCAAT	CAACGGAAAA	CACATCAGCA	TCAAAAACAA
2151	CGGTGGTAAT	GCCGACTTAA	AAAACCTTAA	CGTCCATGCC	AAAAGCGGGG
2201	CATTGAACAT	TCATTCCGAC	CGGGCATTGA	GCATTAGAAA	TACCAAGCTG
2251	GAGTCTACCC	ATAATACGCA	TCTTAATGCA	CAACACGAGC	GGGTAACTGCT
2301	CAACCAAGTA	GATGCCTACG	CACACCGTCA	TCTAAGCATT	ACCGGCAGCC
2351	AGATTTGGCA	AAACGACAAA	CTGCCTTCTG	CCAACAAGCT	GGTGGCTAAC
2401	GGTGTATTGG	CACCTAATGC	GCGCTATTCC	CAAAATGCCG	ACAACACCAC
2451	GCTGAGAGCG	GGTGCAATCA	ACCTTACTGC	CGGTACCGCC	CTAGTCAAGC
2501	GCGGCAACAT	CAATTGGAGT	ACCGTTTCGA	CCAAAACCTT	GGAAGATAAT
2551	GCCGAATTAA	AACCATTGGC	CGGACGGCTG	AATATTGAAG	CAGGTAGCGG
2601	CACATTAACC	ATCGAACCTG	CCAACCGCAT	CAGTGCGCAT	ACCGACCTGA
2651	GCATCAAAAC	AGGCGGAAAA	TTGCTGTTGT	CTGCAAAAGG	AGGAAATGCA
2701	GGTGCGCCTA	GTGCTCAAGT	TTCTTCATTG	GAAGCAAAAG	GCAATATCCG
2751	TCTGGTTACA	GGAGAAACAG	ATTTAAGAGG	TTCTAAAATT	ACAGCCGGTA
2801	AAAATTGGT	TGTCGCCACC	ACCAAAGGCA	AGTTGAATAT	CGAAGCCGTA
2851	AACAATCAT	TCAGCAATTA	TTTTCTTACA	CAAAAAGCGG	CTGAACTCAA
2901	CCAAAATCC	AAAGAATTGG	AACAGCAGAT	TGCGCAGTTG	AAAAAAGCT
2951	CGCCTAAAAG	CAAGCTGATT	CCAACCTTGC	AAGAAGAACG	CGACCGTCTC
3001	GCTTTCTATA	TTCAAGCCAT	CAACAAGGAA	GTTAAAGGTA	AAAAACCCAA
3051	AGGCAAAGAA	TACCTGCAAG	CCAAGCTTTC	TGCACAAAAT	ATTGACTTGA
3101	TTTCCGCACA	AGGCATCGAA	ATCAGCGGTT	CCGATATTAC	CGCTTCCAAA
3151	AAACTGAACC	TTCACGCCGC	AGGCGTATTG	CCAAAGGCAG	CAGATTTCAGA
3201	GGCGGCTGCT	ATTCTGATTG	ACGGCATAAC	CGACCAATAT	GAAATTGGCA
3251	AGCCACCTA	CAAGAGTCAC	TACGACAAAG	CTGCTCTGAA	CAAGCCTTCA
3301	CGTTTGACCG	GACGTACAGG	GGTAAGTATT	CATGCAGCTG	CGGCACTCGA
3351	TGATGCACGT	ATTATTATCG	GTGCATCCGA	AATCAAAGCT	CCCTCAGGCA
3401	GCATAGACAT	CAAGCCCAT	AGTGATATTG	TACTGGAGGC	TGGACAAAAC
3451	GATGCCTATA	CCTTCTTAAA	AACCAAAGGT	AAAAGCGGCA	AAATCATCAG
3501	AAAAACCAAG	TTTACCAGCA	CCCGCGACCA	CCTGATTATG	CCAGCCCCCG
3551	TCGAGCTGAC	CGCCAACGGC	ATAACGCTTC	AGGCAGGCGG	CAACATCGAA
3601	GCTAATACCA	CCCGCTTCAA	TGCCCCTGCA	GGTAAAGTTA	CCCTGGTTGC
3651	GGGTGAAGAG	CTGCAACTGC	TGGCAGAAGA	AGGCATCCAC	AAGCACGAGT
3701	TGGATGTCCA	AAAAAGCCGC	CGCTTTATCG	GCATCAAGGT	AGGCAAGAGC
3751	AATTACAGTA	AAAACGAACT	GAACGAAACC	AAATTGCCTG	TCCGCGTCGT
3801	CGCCCAAAC	GCAGCCACCC	GTTCAGGCTG	GGATACCGTG	CTCGAAGGTA
3851	CCGAATTCAA	AACCACGCTG	GCCGGTGCGG	ACATTACAGC	AGGTGTAGGC
3901	GAAAAAGCCC	GTGCCGATGC	GAAAAATTATC	CTCAAAGGCA	TTGTGAACCG
3951	TATCCAGTCG	GAAGAAAAAT	TAGAAACCAA	CTCAACCGTA	TGGCAGAAAC
4001	AGGCCGGACG	CGGCAGCACT	ATCGAAACGC	TGAAACTGCC	CAGCTTCGAA
4051	AGCCCTACTC	CGCCCAAAC	GACCGCCCCC	GGTGGCTATA	TCGTGCACAT
4101	TCCGAAAGGC	AATTTGAAAA	CCGAAATCGA	AAAGCTGGCC	AAACAGCCCCG
4151	AGTATGCCTA	TCTGAAACAG	CTCCAAGTAG	CGAAAAACGT	CAACTGGAAC
4201	CAGGTGCAAC	TGGCTTACGA	TAAATGGGAC	TATAAGCAGG	AAGGCTTAAC
4251	CAGAGCCGGT	GCAGCGATTG	TTACCATAAT	CGTAACCGCA	CTGACTTATG
4301	GATACGGCGC	AACCGCAGCG	GGCGGTGTAG	CCGCTTCAGG	AAGTAGTACA
4351	GCCGCAGCTG	CCGGAACAGC	CGCCACAACG	ACAGCAGCAG	CTACTACCGT
4401	TTCTACAGCG	ACTGCCATGC	AAACCGCTGC	TTTAGCCTCC	TTGTATAGCC
4451	AAGCAGCTGT	ATCCATCATC	AATAATAAAG	GTGATGTCGG	CAAAGCGTTG
4501	AAAGATCTCG	GCACCAAGTGA	TACGGTCAAG	CAGATTGTCA	CTTCTGCCCT
4551	GACGGCGGGT	GCATTAAATC	AGATGGGCGC	AGATATTGCC	CAATTGAACA
4601	GCAAGGTAAG	AACCGAACTG	TTCAGCAGTA	CGGGCAATCA	AACTATTGCC
4651	AACCTTGGAG	ACCACTGGC	TACCAATCTC	AGTAATGCAG	GTATCTCAGC
4701	TGGTATCAAT	ACCGCCGTCA	ACGGCGGCAG	CCTGAAAGAC	AACTTAGGCA
4751	ATGCCGCATT	AGGAGCATTG	GTTAATAGCT	TCCAAGGAGA	AGCCGCCAGC
4801	AAAATCAAAA	CAACCTTCAG	CGACGATTAT	GTTGCCAAAC	AGTTCGCCCA

	4851	CGCTTTGGCT	GGGTGTGTTA	GCGGATTGGT	ACAAGGAAAA	TGTAAAGACG
	4901	GGGCAATTGG	CGCAGCAGTT	GGGGAAATCG	TAGCCGACTC	CATGCTTGGC
	4951	GGCAGAAACC	CTGCTACACT	CAGCGATGCG	GAAAAGCATA	AGGTTATCAG
5	5001	TTACTCGAAG	ATTATTGCCG	GCAGCGTGGC	GGCACTCAAC	GGCGCGGATG
	5051	TGAATACTGC	GGCGAATGCG	GCTGAGGTGG	CGGTAGTGAA	TAATGCTTTG
	5101	AATTTTGACA	GTACCCCTAC	CAATGCGAAA	AAGCATCAAC	CGCAGAAGCC
	5151	CGACAAAACC	GCACTGGAAA	AAATTATCCA	AGGTATATG	CCTGCACATG
	5201	CAGCAGGTGC	GATGACTAAT	CCGCAGGATA	AGGATGCTGC	CATTTGGATA
10	5251	AGCAATATCC	GTAATGGCAT	CACAGGCCCG	ATTGTGATTA	CCAGCTATGG
	5301	GGTTTATGCT	GCAGGTTGGA	CAGCTCCGCT	GATCGGTACA	GCGGGTAAAT
	5351	TAGCTATCAG	CACCTGCATG	GCTAATCCTT	CTGGTTGTAC	TGTCATGGTC
	5401	ACTCAGGTG	CCGAAGCGGG	CGCGGGAATC	GCCACGGGTG	CGGTAACGGT
	5451	AGGCAACGCT	TGGGAAGCGC	CTGTGGGGGC	GTTGTGCGAAA	GCGAAGGCGG
15	5501	CCAAGCAGGC	TATACCAACC	CAGACAGTTA	AAGAACTTGA	TGGCTTACTA
	5551	CAAGAATCAA	AAAATATAGG	TGCTGTAAAT	ACACGAATTA	ATATAGCGAA
	5601	TAGTACTACT	CGATATACAC	CAATGAGACA	AACGGGACAA	CCGGTATCTG
	5651	CTGGCTTTGA	GCATGTTCTT	GAGGGGCACT	TCCATAGGCC	TATTGCGAAT
	5701	AACCGTTCAG	TTTTTACCAT	CTCCCCAAAT	GAATTGAAGG	TTATACTTCA
20	5751	AAGTAATAAA	GTAGTTTCTT	CTCCCGTATC	GATGACTCCT	GATGGCCAAT
	5801	ATATGCGGAC	TGTCGATGTA	GGAAAAGTTA	TTGGTACTAC	TTCTATTAAA
	5851	GAAGGTGGAC	AACCCACAAC	TACAATTAAA	GTATTTACAG	ATAAGTCAGG
	5901	AAATTTGATT	ACTACATACC	CAGTAAAGG	AAACTAA	

This corresponds to the amino acid sequence <SEQ ID 60; ORF114-1>:

	1	MNKGHLRIIF	SKKHSTMVAV	AETANSQKGK	KQAGSSVSVS	LKTSGLDLCK
25	51	LKTTLLKTLVC	SLVSLSMVLP	AHAQITTDKS	APKNQQVVIL	KTNTGAPLVN
	101	IQTPNGRGLS	HNRYTQFDVD	NKGAVLNNDR	NNNPFVVKGS	AQLILNEVRG
	151	TASKLNGIVT	VGGQKADVII	ANPNGITVNG	GGFKNVGRGI	LTTGAPQIGK
	201	DGALTGFVDV	QGTLLTVGAAG	WNDKGGADYT	GVLARAVALQ	GKLGQKNLAV
	251	STGPQKVDYA	SGEISAGTAA	GTKPTIALDT	AALGGMYADS	ITLIANEKGV
30	301	GVKNAGTLEA	AKQLIVTSSG	RIENSGRIAT	TADGTEASPT	YLSIETTEKG
	351	AAGTFISNGG	RIESKGLLVI	ETGEDISLRN	GAVVQNNNGSR	PATTVLNAGH
	401	NLVIESKTNV	NNAKGPATLS	ADGRTVIKEA	SIQTGTTVYS	SSKGNAELGN
	451	NTRITGADV	VLSNGTISSS	AVIDAKDTAH	IEAGKPLSLE	ASTVTSDIRL
	501	NGGSIKGGKQ	LALLADDNIT	AKTTNLNTPG	NLYVHTGKDL	NLNVDKDLA
35	551	ASIHLSKSDNA	AHITGTSKTL	TASKDMGVEA	GSLNVTNTNL	RTNSGNLHIQ
	601	AAKGNLQLRN	TKLNAAKALE	TTALQGNIVS	DGLHAVSADG	HVSLLANGNA
	651	DFTGHNTLTA	KADVNAAGSV	KGRLKADNTN	ITSSSGDITL	VAGNGIQLGD
	701	GKSNRSINGK	HISIKNNGGN	ADLKNLNVHA	KSGALNIHSD	RALSIENTKL
40	751	ESTHNTLHNA	QHERVTLNQV	DAYAHRHLSI	TGSQIWQNDK	LPSANKLVAN
	801	GVLAALNARYS	QIADNTTLRA	GAINLTAGTA	LVKRGININWS	TVSTKTLEDN
	851	AELKPLAGRL	NIEAGSGTTL	IEPANRISAH	TDLSIKTGK	LLLSAKGGNA
	901	GKSAQVSSL	EAKGNIRLVT	GETDLRSGKI	TAGKNLVVAT	TKGKLNIEAV
	951	NNSFSNYFPT	QKAAELNQKS	KELEQQIAQL	KKSSPKSKLI	PTLQEERDRL
45	1001	AFYIQAINK	VKGKKPKGKE	YLQAKLSAQN	IDLISAQGIE	ISGSDITASK
	1051	KLNLHAAGVL	PKAADSEAAA	ILIDGITDQY	EIGKPTYKSH	YDKAALNKPS
	1101	RLTGRTGVSI	HAAAALDDAR	IIIGASEIKA	PSGSIDIKAH	SDIVLEAGQN
	1151	DAYTFLKTKG	KSGKIIIRTK	FTSTRDHLIM	PAPVELTANG	ITLQAGGNIE
	1201	ANTTRFNAPA	GKVTLVAGEE	LQLLAEEGIH	KHELDVQKSR	RFIGIKVGKS
50	1251	NYSKNEINET	KLPVRVVAQT	AATRSWDTV	LEGTEFKTTL	AGADIQAGVG
	1301	EKARADAKII	LKGIVNRIQS	EKLETNSTV	WQKQAGRGST	IETLKLPSFE
	1351	SPTPPKLTAP	GGYIVDIPKG	NLKEIEKLA	KQPEYAYLKQ	LQVAKNVNWN
	1401	QVQLAYDKWD	YKQEGLTRAG	AAIVTIIIVTA	LTYGYGATAA	GGVAASGSST
	1451	AAAAGTAATT	TAAATTVSTA	TAMQTAALAS	LYSQAQVSI	NNKGDVGKAL
55	1501	KDLGTSDTVK	QIVTSALTAG	ALNQMGADIA	QLNSKVRTEL	FSSTGNQTIA
	1551	NLGGRLATNL	SNAGISAGIN	TAVNGGSLKD	NLGNALGAL	VNSFQGEAAS
	1601	KIKTTFSDDY	VAKQFAHALA	GCVSGLVQGK	CKDGAIGAAV	GEIVADSMGL
	1651	GRNPATLSDA	EKHKVISYSK	IIAGSVAALN	GGDVNTAANA	AEVAVVNNAL
	1701	NFDSTPTNAK	KHQPQKPKDKT	ALEKIIQGIM	PAHAAGAMTN	PQDKDAAIWI
60	1751	SNIRNGITGP	IVITSYGVYA	AGWTAPLIGT	AGKLAISTCM	ANPSGTVMV
	1801	TQAAEAGAGI	ATGAVTVGNA	WEAPVGALSK	AKAAKQAIPT	QTVKELDGLL
	1851	QESKNIGAVN	TRINIANSTT	RYTPMRQTGQ	PVSAGFEHVL	EGHFHRIPIAN
	1901	NRSVFTISPN	ELKVILQSNK	VVSSPVSMTF	DGQYMRTVDV	GKVIQTTSIK
	1951	EGGQPTTTIK	VFTDKSGNLI	TTYPVKGN*		

Computer analysis of this amino acid sequence predicts a transmembrane region and also gives the

following results:

Homology with a predicted ORF from *N.meningitidis* (strain A)

ORF114 shows 91.9% identity over a 284aa overlap with an ORF (ORF114a) from strain A of *N. meningitidis*:

5	orf114.pep	10	20	30	40
		AVAETANSQKGKQAGSSVS	SLKTS	SGDLCGK	LKTLVLC
	orf114a	MNKGLHRIIFS	KKHSTMVAVAETANSQKGKQAGSSVS	SLKTS	SGDLCGK
		10	20	30	40
10	orf114.pep	50	60	70	80
		SLVSLSMVLP	PAHAQITTDKS	APKNQVVIL	KTN
	orf114a	SLVSLSMXXXXX	QITTDKS	APKNQVVIL	KTN
		70	80	90	100
15	orf114.pep	110	120	130	140
		NKGAVLNND	RNNPFVVK	GSAQLIL	NEVRGT
	orf114a	NKGAVLNND	RNNPFVVK	GSAQLIL	NEVRGT
		130	140	150	160
20	orf114.pep	170	180	190	200
		GGFKNVGR	GILTTGAP	QIGKDG	ALTGF
	orf114a	GGFKNVGR	GILTTGAP	QIGKDG	ALTGF
		190	200	210	220
25	orf114.pep	230	240	250	260
		GKXXGKXL	AVSTGPQ	KVDYAS	GEISAG
	orf114a	GKXXGKXL	AVSTGPQ	KVDYAS	GEISAG
		250	260	270	280
30	orf114.pep	310	320	330	340
		GVX			
	orf114a	GVKNAGT	LEAAKQL	IIVTSS	GRIENSG
		310	320	330	340

The complete length ORF114a nucleotide sequence <SEQ ID 61> is:

40	1	ATGAATAAAG	GTTTACATCG	CATTATCTTT	AGTAAAAAGC	ACAGCACCAT
	51	GGTTGCAGTA	GCCGAAACTG	CCAACAGCCA	GGGCAAAGGT	AAACAGGCAG
	101	GCAGTTCGGT	TTCTGTTTCA	CTGAAACTT	CAGGCGACCT	TTGCGGCAAA
	151	CTCAAAACCA	CCCTTAAAC	CTTGGTCTGC	TCTTTGGTTT	CCCTGAGTAT
45	201	GGNATTNCNN	NNCNNTNCCC	AAATTACCAC	CGACAAATCA	GCACCTAAAA
	251	ACCANCAGGT	CGTTATCCTT	AAAACCAACA	CTGGTGCCCC	CTGGTGAAT
	301	ATCCAAACTC	CGAATGGACG	CGGATTGAGC	CACAACCGCT	ATACGCAGTT
	351	TGATGTTGAC	AACAAAGGGG	CAGTGTAAAA	CAACGACCGT	AACAATAATC
	401	CGTTTCTGGT	CAAAGGCAGT	GCGCAATTGA	TTTTGAACGA	GGTACGCGGT
	451	ACGGCTAGCA	AACTCAACGG	CATCGTTACC	GTAGGCGGTC	AAAAGGCCGA
50	501	CGTGATTATT	GCCAACCCCA	ACGGCATTAC	CGTTAATGGC	GGCGGCTTTA
	551	AAAATGTCGG	TCGGGGCATC	TTAACTATCG	GTGCGCCCCA	AATCGGCAAA
	601	GACGGTGAC	TGACAGGATT	TGATGTGCGT	CAAGGCACAT	TGACCGTAGG
	651	AGCAGCAGGT	TGGAATGATA	AAGGCGGAGC	CGACTACACC	GGGTACTTTG
	701	CTCGTGAGT	TGCTTTGCAG	GGGAAATTAC	AGGGTAAAAA	CCTGGCGGTT
55	751	TCTACCGGTC	CTCAGAAAGT	AGATTACGCC	AGCGGCGAAA	TCAGTGCAGG
	801	TACGGCAGCG	GGTACGAAAC	CGACTATTGC	CCTTGATACT	GCCGCACTGG
	851	GCGGTATGTA	CGCCGACAGC	ATCACACTGA	TTGCCANTGA	AAAAGGCGTA
	901	GGCGTCAAAA	ATGCCGGCAC	ACTCGAAGCG	GCCAAGCAAT	TGATTGTGAC
	951	TTCTGTCAGG	CGCATTGAAA	ACAGCGGCCG	CATCGCCACC	ACTGCCGACG
60	1001	GCACCGAAGC	TTCACCGACT	TATCTNNCNA	TCGAAACCAC	CGAAAAAGGA
	1051	GCNNCAGGCA	CATTTATCTC	CAATGGTGGT	CGGATCGAGA	GCAAAGGCTT
	1101	ATTGGTTATT	GAGACGGGAG	AAGATATCAN	CTTGCGTAAC	GGAGCCGTGG
	1151	TGCAGAATAA	CGGCAGTCGC	CCAGCTACCA	CGGTATTAAA	TGCTGGTCAT
	1201	AATTTGGTGA	TTGAGAGTAA	AACTAATGTG	AACAATGCCA	AAGGCTCGNC

1251 TAATCTGTCTG GCCGGCGGTC GTACTACGAT CAATGATGCT ACTATTCAAG  
 1301 CGGGCAGTTC CGTGTACAGC TCCACCAAAG GCGATACTGA NTTGGGTGAA  
 1351 AATACCCGTA TTATTGCTGA AAACGTAACC GTATTATCTA ACGGTAGTAT  
 1401 TGGCAGTGCT GCTGTAATTG AGGCTAAAGA CACTGCACAC ATTGAATCGG  
 1451 GCAAACCGCT TTCTTTAGAA ACCTCGACCG TTGCCTCCAA CATCCGTTTG  
 1501 AACAACGGTA ACATTAAAGG CGGAAAGCAG CTTGCTTTAC TGGCAGACGA  
 1551 TAACATTACT GCCAAAACCTA CCAATCTGAA TACTCCCGGC AATCTGTATG  
 1601 TTCATACAGG TAAAGATCTG AATTTGAATG TTGATAAAGA TTTGCTGTGCC  
 1651 GCCAGCATCC ATTTGAAATC GGATAACGCT GCCCATATTA CCGGCACCAG  
 1701 TAAAACCCCTC ACTGCCTCAA AAGACATGGG TGTGGAGGCA GGCTTGCTGA  
 1751 ATGTTACCAA TACCAATCTG CGTACCAACT CGGGTAATCT GCACATTGAG  
 1801 GCAGCCAAAG GCAATATTCA GCTTCGCAAT ACCAAGCTGA ACGCAGCCAA  
 1851 GGCTCTCGAA ACCACCGCAT TGCAGGGCAA TATCGTTTCA GACGGCCTTC  
 1901 ATGCTGTTTC TGCAGACGGT CATGTATCCT TATTGGCCAA CGGTAATGCC  
 1951 GACTTTACCG GTCACAATAC CCTGACAGCC AAGGCCGATG TCNATGACAG  
 2001 ATCGGTTGGT AAAGGCCGTC TGAAAGCAGA CAATACCAAT ATCACTTCAT  
 2051 CTTCAGGAGA TATTACGTTG GTTGCCGNNN NCGGTATTCA GCTTGGTGAC  
 2101 GGAAAACAAC GCAATTCAAT CAACGGAAAA CACATCAGCA TCAAAAACAA  
 2151 CGGTGGTAAT GCCGACTTAA AAAACCTTAA CGTCCATGCC AAAAGCGGGG  
 2201 CATTTGAACAT TCATTCCGAC CGGGCATTGA GCATAGAAAA TACNAAGCTG  
 2251 GAGTCTACCC ATAATCGCA TCTTAATGCA CAACACGAGC GGGTAACGCT  
 2301 CAACCAAGTA GATGCCTACG CACACCGTCA TCTAAGCATT ANCGGCAGCC  
 2351 AGATTTGGCA AAACGACAAA CTGCCTTCTG CCAACAAGCT GGTGGCTAAC  
 2401 GGTGTATTGG CANTCAATGC GCGCTATTCC CAAATTGCGG ACAACACCAC  
 2451 GCTGAGAGCG GGTGCAATCA ACCTTACTGC CGGTACCGCC CTAGTCAAGC  
 2501 GCGGCAACAT CAATTGGAGT ACCGTTTCGA CCAAGACTTT GGAAGATAAT  
 2551 GCCGAATTAA AACCATTGGC CGGACGGCTG AATATTGAAG CAGGTAGCGG  
 2601 CACATTAACC ATCGAACCTG CCAACCGCAT CAGTGCGCAT ACCGACCTGA  
 2651 GCATCAAAAC AGGCGGAAAA TTGCTGTTGT CTGCAAAAGG AGGAAATGCA  
 2701 GGTGCGCNTA GTGCTCAAGT TTCTCATTG GAAGCAAAAG GCAATATCCG  
 2751 TCTGGTTACA GGAGNAACAG ATTTAAGAGG TTCTAAATT ACAGCCGGTA  
 2801 AAAAATTGGT TGTGCGCCACC ACCAAAGGCA AGTTGAATAT CGAAGCCGTA  
 2851 AACAACTCAT TCAGCAATTA TTTTCNTACA CAAAAAGNGN NNGNNCTCAA  
 2901 CCAAAAATCC AAAGAATTGG AACAGCAGAT TGCGCAGTTG AAAAAAGCT  
 2951 CGCNTAAAAG CAAGCTGATT CCAACCCTGC AAGAAGAACG CGACCGTCTC  
 3001 GCTTTCTATA TTCAAGCCAT CAACAAGGAA GTTAAAGGTA AAAAACCCAA  
 3051 AGGCAAAAGAA TACCTGCAAG CCAAGCTTTC TGCACAAAAT ATTGACTTGA  
 3101 TTTCCGCACA AGGCATCGAA ATCAGCGGTT CCGATATTAC CGCTTCCAAA  
 3151 AAAGTGAACC TTCACGCCGC AGGCGTATTG CCAAAGGCAG CAGATTGAGA  
 3201 GCGCGCTGCT ATTCTGATTG ACGGCATAAC CGACCAATAT GAAATTGCGA  
 3251 AGCCCACTCA CAAGAGTCAC TACGACAAAG CTGCTCTGAA CAAGCCTTCA  
 3301 CGTTTGACCG GACGTACGGG GGTAAGTATT CATGCAGCTG CGGCACTGCA  
 3351 TGATGCACGT ATTATTATCG GTGCATCCGA AATCAAAGCT CCCTCAGGCA  
 3401 GCATAGACAT CAAAGCCCAT AGTGATATTG TACTGGAGGC TGGACAAAAC  
 3451 GATGCCTATA CCTTCTTANA AACCAAAGGT AAAAGCGGCA NAATNATCAG  
 3501 AAAAAACNAAG TTTACCAGCA CCNGCGANCA CCTGATTATG CCAGGCCNG  
 3551 TCGAGCTGAC CGCCAACGGT ATCACGCTTC AGGCAGGCGG CAACATCGAA  
 3601 GCTAATACCA CCCGCTTCAA TGCCCTGCA GGTAAGGTTA CCCTGGTTGC  
 3651 GGGTGAANAG NTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT  
 3701 TGGATGTCCA AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGTNAGAGC  
 3751 AATTACAGTA AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT  
 3801 CGCCCAAANT GCAGCCACCC GTTCAGGCTG GGATACCGTG CTCGAAGGTA  
 3851 CCGAATTCAA AACCACGCTG GCCGGTGCCG ACATTGAGGC AGGTGTANGC  
 3901 GAAAAAGCCC GTGTCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG  
 3951 TATCCAGTCG GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC  
 4001 AGGCCGGACG CGGCAGCACT ATCGAAACGC TAAAGCTGCC CAGCTTCGAA  
 4051 AGCCCTACTC CGCCCAAATT GTCCGCACCC GGCGGNTATA TCGTCGACAT  
 4101 TCCGAAAGGC AATCTGAAAA CCGAAATCGA AAAGCTGTCC AAACAGCCCG  
 4151 AGTATGCCTA TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT  
 4201 CAGGTGCAGC TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC  
 4251 CGAAGCAGGT GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG  
 4301 GCGCAGGAAC CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA  
 4351 ACGGATGCAG CATTGCGCTC TTTGGCCAGC CAGGCTTCCG TATCGTTTAT  
 4401 CAACAACAAA GGCGATGTCT GCAAAACCCCT GAAAGAGCTG GGCAGAAGCA  
 4451 GCACGGTGAA AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC  
 4501 AAAATCGGCG CTTGCGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA  
 4551 CAACCTGACC GTCAACCTAG CCAATGNCAG GCAGTGCCGC ACTGATTaa

This encodes a protein having amino acid sequence <SEQ ID 62>:

1 MNKGLHRIIF SKKHSTMVAV AETANSQKGK KQAGSSSVSVS LKTSGLDLCGK

5 51 LKTTTLKTLVC SLVSLSMXXX XXXQITTDKS APKNXQVVIL KTNTGAPLVN  
 101 IQTPNGRGLS HNRYTQFDVD NKGAVLNNDNR NNNPFLVKGS AQLILNEVRG  
 151 TASKLNGIVT VGGQKADVII ANPNGITVNG GGFKNVGRGI LTIGAPQIGK  
 201 DGALTGFVDVR QGTLTVGAAG WNDKGGADYT GVLARAVALQ GKLGQKNLAV  
 251 STGPQKVDYA SGEISAGTAA GTKPTIALDT AALGGMYADS ITLIAXEKGV  
 301 GVKNAGTLEA AKQLIVTSSG RIENSGRIAT TADGTEASPT YLXIETTEKG  
 351 AXGTFISNGG RIESKGLLVI ETGEDIXLRN GAVVQNNNGSR PATTVLNAGH  
 401 NLVIESKTNV NNAKGSXNLS AGGRTTINDA TIQAGSSVYS STKGD TXLGE  
 10 451 NTRIIAENV T VLSNGSIGSA AVIEAKDTAH IESGKPLSLE TSTVASNIRL  
 501 NNGNIKGGKQ LALLADDNIT AKTTNLNTPG NLYVHTGKDL NLNVDKDLSA  
 551 ASIHLSKSDNA AHITGTSKTL TASKDMGVEA GLLNVTNTNL RTNSGNLHIQ  
 601 AAKGNIQLRN TKLNAAKALE TTALQGNIVS DGLHAVSADG HVSLLANGNA  
 651 DFTGHNTLTA KADVXAGSVG KGRLKADNTN ITSSSGDITL VAXXGIQLGD  
 701 GKQRNSINGK HISIKNNGGN ADLKNLNVHA KSGALNIHSD RALSIENTKL  
 15 751 ESTHNTHLNA QHERVTLNQV DAYAHRHLSI XGSQIWQNDK LPSANKLVAN  
 801 AFLIXNARYS QIADNTTLRA GAINLTAGTA LVKRGNNINWS TVSTKTLEDN  
 851 AELKPLAGRL NIEAGSGTTL IEPANRISAH TDLSTKTGGK LLLSAKGNNA  
 901 GAXSAQVSSL EAKGNIRLVT GXTDLRGSKI TAGKNLVVAT TKGKLNIEAV  
 951 NNSFSNYFXT QKXXXLNQKS KELEQQIAQL KKSSXKSKLI PTLQOEERDL  
 20 1001 AFYIQAINKE VKGKKPKGKE YLQAKLSAQN IDLISAQIE ISGSDITASK  
 1051 KLNLAAGVL PKAADSEAAA ILIDGITDQY EIGKPTYKSH YDKAALNPKS  
 1101 RLTGRTGVSI HAAAALDDAR IIGASEIKA PSGSIDIKAH SDIVLEAGQN  
 1151 DAYTFLXTKG KSGXXIRKTK FTSTXXHLIM PAPVELTANG ITLQAGNIE  
 25 1201 ANTTRFNAPA GKVTLVAGEX XQLLAEEGHI KHELDVQKSR RFIGIKVXGS  
 1251 NYSKNEINET KLPVRVVAQX AATRSWDTV LEGTEFKTTL AGADIQAGVX  
 1301 EKARVDAKII LKGIVNRIQS EEKLETNSTV WQKQAGRGST IETLKLPSFE  
 1351 SPTPKLSAP GGYIVDIPKG NLKTEIEKLS KQPEYAYLKO LQVAKNINWN  
 1401 QVQLAYDRWD YKQEGLTEAG AAIALAVTV VTSAGGTGAV LGLNGAXAAA  
 1451 TDAAFASLAS QASVSFINNK GDVGKTLKEL GRSSVTKNLV VAAATAGVAD  
 30 1501 KIGASALXNV SDKQWINNLT VNLANXGQCR TD\*

ORF114-1 and ORF114a show 89.8% identity in 1564 aa overlap

35 orf114a.pep MNKGLHRIIFSCKKHSTMVAVAETANSQKGKQAGSSVSLSKTSGLCGKLTTLKTLVC  
 orf114-1 MNKGLHRIIFSCKKHSTMVAVAETANSQKGKQAGSSVSLSKTSGLCGKLTTLKTLVC  
 40 orf114a.pep SLVSLSMXXXXXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTNGRGLSHNRYTQFDVD  
 orf114-1 SLVSLSMVLPAAHAQITTDKSAPKNQQVVILKTNTGAPLVNIQTNGRGLSHNRYTQFDVD  
 45 orf114a.pep NKGAVLNNDNRNNNPFLVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG  
 orf114-1 NKGAVLNNDNRNNNPFLVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG  
 50 orf114a.pep GGFKNVGRGILTIGAPQIGKDGALTGFVDVRQGTTLTVGAAGWNDKGGADYTGVLARAVALQ  
 orf114-1 GGFKNVGRGILTIGAPQIGKDGALTGFVDVRQGTTLTVGAAGWNDKGGADYTGVLARAVALQ  
 55 orf114a.pep GKLGQKNLAVSTGPQKVDYASGEISAGTAAAGTKPTIALDTAALGGMYADSITLIAXEKGV  
 orf114-1 GKLGQKNLAVSTGPQKVDYASGEISAGTAAAGTKPTIALDTAALGGMYADSITLIANEKGV  
 60 orf114a.pep GVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXGTFISNGG  
 orf114-1 GVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLSIETTEKGAAGTFISNGG  
 65 orf114a.pep RIESKGLLVIETGEDIXLRNGAVVQNNNGSRPATTVLNAGHNLVIESKTNVNNNAKGSXNLS  
 orf114-1 RIESKGLLVIETGEDISLRNGAVVQNNNGSRPATTVLNAGHNLVIESKTNVNNNAKGPATLS  
 70 orf114a.pep AGGRTTINDATIQAGSSVYSSTKGD TXLGENTRIIAENVTVLSNGSIGSAAVIEAKDTAH  
 orf114-1 ADGRTVIKEASIQGTTVYSSSKGNAELGNNTTRITGADVTVLSNGTISSSAVIDAKDTAH  
 75 orf114a.pep IESGKPLSLETSTVASNIRLNNNGNIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL  
 orf114-1 IEAGKPLSLEASTVTSDIRLNGGSIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL  
 80 orf114a.pep NLNVDKDLSAASIHLSKSDNAAHITGTSKTLTASKDMGVEAGLLNVTNTNLRTNSGNLHIQ  
 orf114-1 NLNVDKDLSAASIHLSKSDNAAHITGTSKTLTASKDMGVEAGLLNVTNTNLRTNSGNLHIQ

	orf114-1	NLNVDKDLSAASIHLKSDNAAHITGTSTLTASKDMGVEAGSLNVTNTNLRTNSGNLHIQ	
	orf114a.pep	AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNAFTGHNTLTA	
5	orf114-1	AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNAFTGHNTLTA	
	orf114a.pep	KADVXAGSVGKGRLLKADNTNITSSSGDITLVAXXGIQLGDGKQRNSINGKHHISIKNNGGN	
10	orf114-1	KADVNAGSVGKGRLLKADNTNITSSSGDITLVAGNGIQLGDGKQRNSINGKHHISIKNNGGN	
	orf114a.pep	ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI	
	orf114-1	ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI	
15	orf114a.pep	XGSQIWQNDKLP SANKLVANGVLAXNARYSQIADNTTLRAGAINLTAGTALVKGNGINWS	
	orf114-1	TGSQIWQNDKLP SANKLVANGVLALNARYSQIADNTTLRAGAINLTAGTALVKGNGINWS	
20	orf114a.pep	TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGKLLLSAKGGNA	
	orf114-1	TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGKLLLSAKGGNA	
	orf114a.pep	GAXSAQVSSLEAKGNIRLVGTGXTDLRSGKITAGKNLVVATTGKGLNIEAVNNSFSNYFXT	
25	orf114-1	GAPSAQVSSLEAKGNIRLVGTGETDLRSGKITAGKNLVVATTGKGLNIEAVNNSFSNYFPT	
	orf114a.pep	QKXXXLNQKSKELEQQIAQLKKSSXKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE	
30	orf114-1	QKAAELNQKSKELEQQIAQLKKSSPKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE	
	orf114a.pep	YLQAKLSAQNIDLISAQGIEISGSDITASKKLNLAAGVLPKAADSEAAAAILIDGITDQY	
	orf114-1	YLQAKLSAQNIDLISAQGIEISGSDITASKKLNLAAGVLPKAADSEAAAAILIDGITDQY	
35	orf114a.pep	EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIGASEIKAPSGSIDIKAH	
	orf114-1	EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIGASEIKAPSGSIDIKAH	
40	orf114a.pep	SDIVLEAGQNDAYTFLXTKGKSGXXIRKTKFTSTXXHLIMPAPVELTANGITLQAGGNIE	
	orf114-1	SDIVLEAGQNDAYTFLKTKGKSGKIIRKTKFTSTRDHLIMPAPVELTANGITLQAGGNIE	
	orf114a.pep	ANTTRFNAPAGKVTLVAGEXXQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNET	
45	orf114-1	ANTTRFNAPAGKVTLVAGEELQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNET	
	orf114a.pep	KLPVRVVAQXAATRSGWDTVLEGTEFKTTLAGADIQAGVXEKARVDAKIILKGIVNRIQS	
50	orf114-1	KLPVRVVAQTAATRSGWDTVLEGTEFKTTLAGADIQAGVGEKARADAKIILKGIVNRIQS	
	orf114a.pep	EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLSAPGGYIVDIPKGNLKTEIEKLS	
	orf114-1	EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLTAPGGYIVDIPKGNLKTEIEKLA	
55	orf114a.pep	KQPEYAYLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSGAGTGAV	
	orf114-1	KQPEYAYLKQLQVAKNVNWNQVQLAYDKWDYKQEGLTRAGAAIVTIIIVTALTYGYGATAA	
60	orf114a.pep	LGLNGA-----XAAATD-----AAFASLASQASVSFINNKGVDVGKTL	1477
	orf114-1	GGVAASGSSTAAAAGTAATTTAAATTVSTATAMQTAALASLYSQAAVSIINNKGVDVGKAL	1500
	orf114a.pep	KELGRSSTVKNLVVAAATAGVADKIGA-----SALXNVSDKQWINNL---TVNL	1523
65	orf114-1	KDLGTSDTVQKIVTSALTAGALNQMGADIAQLNSKVRTELFSSSTGNQTIANLGRRLATNL	1560
	orf114a.pep	ANXGQCRTDX	
	orf114-1	SNAGISAGINTAVN...	
70			

Homology with pspA putative secreted protein of *N.meningitidis* (accession number AF030941)

ORF114 and pspA protein show 36% aa identity in 302aa overlap:

```

5      Orf114: 1   AVAETANSQKGKQAGSSVSLSL-----KTS G DXXXXXXXXXXXXXXXXXXXXXXXXXPAHAQ 56
      pspA: 19   AVAENVHRD G K S M Q D S E A A S V R V T G A A S V S S A R A A F G F R M A A F S V M L A L G V A A F S P A P A S 78

10     Orf114: 57   -ITTDKSAPKNQQVVILKTNTGAPLVNIQTTPNGRGLSHNRXYAFDVDNKGAVLNNDNRNN- 114
      pspA: 79   GIIADKSAPKNQQAVILQ TANGLPQVNIQT P S S Q G V S V N R F K Q F D V D E K G V I L N N S R S N T 138

15     Orf114: 115  -----NPFVVKGS A Q L I L N E V - R G T A S K L N G I V T V G G Q K A D V I I A N P N G I T V N G G 163
      pspA: 139  Q T Q L G G W I Q G N P H L A R G E A R V I V N Q I D S S N P S L L N G Y I E V G G K R A E V V A N P S G I R V N G G 198

20     Orf114: 164  G F K N V G R G I L T T G A P Q I G K D G A L T G F D V V K A H W T V X A A G W N D K G G A X Y T G V L A R A V A L Q G 223
      pspA: 199  G L I N A A S V T L T S G V P V L - N N G N L T G F D V S S G K V V I G G K G L - D T S D A D Y T R I L S R A A E I N A 256

25     Orf114: 224  K X X G K X L A V S T G P Q K V D Y A S G E I S A G T A A G T K - - - - P T I A L D T A A L G G M Y A D S I T L I A N E 279
      pspA: 257  G V W G K D V K V S G K N K L D F D G S L A K T A S A P S S S D S V T P T V A I D T A T L G G M Y A D K I T L I S T D 316

      Orf114: 280  K G 281
      pspA: 317  N G 318

```

ORF114a is also homologous to pspA:

```

30     gi|2623258 (AF030941) putative secreted protein [Neisseria meningitidis] Length
      = 2273
      Score = 261 bits (659), Expect = 3e-68
      Identities = 203/663 (30%), Positives = 314/663 (46%), Gaps = 76/663 (11%)

35     Query: 1   MNKGLHRIIFSKKHSTMVAVAETANSQKGKQAGSSVSLSL-----TSGDXXXXXXXXXX 55
      Sbjct: 1   MNKRCYKVI FNKKRSCMMAVAENVHRD G K S M Q D S E A A S V R V T G A A S V S S A R A A F G F R M A A 60

40     Query: 56  XXXXXXXXXXXXXXXXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTTPNGRGLSHNRYT 115
      Sbjct: 61  FSVMLALGVA AFSPAPASGIIADKSAPKNQQAVILQ TANGLPQVNIQT P S S Q G V S V N R F K 120

45     Query: 116 QFDVDNKGAVLNNDNRNN-----NPFVVKGS A Q L I L N E V - R G T A S K L N G I V T V G G 163
      Sbjct: 121 QFDVDEKGVILNNSRSNTQTQLGGW IQGNPHLARGEARVIVNQIDSSNP S L L N G Y I E V G G 180

50     Query: 164 QKADVIIANPNGITVNGGGFKNVGRGILTIGAPQIGKD GALTGF D V R Q G T L T V G A A G W N D 223
      Sbjct: 181 KRAEVVVANPSGIRVNGGGLINAASVTLTSGVPVL-NNGNLTGF D V S S G K V V I G G K G L - D 238

55     Query: 224 KGGADYTGV L A R A V A L Q G K L Q G K N L A V S T G P Q K V D Y A S G E I S A G T A A G T K - - - - P T I A L D 279
      Sbjct: 239 TSDADYTRILSRAAEINAGVWGKDVKVVS G K N K L D F D G S L A K T A S A P S S S D S V T P T V A I D 298

60     Query: 280 T A A L G G M Y A D S I T L I A X E K G V G V K N A G T L E A A K - Q L I V T S S G R I E N S G R I A T T A D G T E A S 338
      Sbjct: 299 T A T L G G M Y A D K I T L I S T D N G A V I R N K G R I F A A T G G V T L S A D G K L S N S G S I - - - - - D A A 351

65     Query: 339 P T Y L X I E T T E K G A X G T F I S N G G R I E S K G L L V I E T G E D I X L R N G A V V Q N N G S R P A T T V L N A 398
      Sbjct: 352 E I T I S A Q T V D - - - - - N R Q G F I R S G K G S V L K V S D G I N N Q A G L I - - - - G S A G L L D I R D T 399

      Query: 399 G H N L V I E S K T N V N N A K S - - - - X N L S A G G R T T I N D A T I Q A G S S V S S T K G D T X L G E N T R I 454
      Sbjct: 400 G - - - - K S S L H I N N T D G T I I A G K D V S L Q A K S L D N D G I L T A A R D V - S V S L H D D F A G K R D I E 453

      Query: 455 I A E N V T V L S N G S I G S A A V I E A K D T A H I E S G K P L S L E T S T V A S N I R L N N G N I K G G K Q L A L L 514
      Sbjct: 455 + T + G + + + I + A D T + + + + + S R G L +

```

Sbjct: 454 AGRTLTFSTQGRLLKNTRIIQAGDTVSLTAAQIDNTVSGKIQSGNRTGLNGKNGITNRGLI 513  
 Query: 515 ADDNIT-----AKTTNLNTPGNLYVHTGKDLNLNVDKDLASAASIHLSKDAAHITGTSKT 569  
 + IT AK+ N T G +Y G + + D L+ AA  
 Sbjct: 514 NSNGITLLQTEAKSDNAGT-GRIY---GSRVAVEADTLLNREETVNGETKAA-----V 562  
 Query: 570 LTASKDMGVEAGXXXXXXXXXXXXSGNLHIQAA---KGNIQLRNTKL-NAAKALETALQ 625  
 + A + + + A SG+LHI +A +Q NT L N + A+E++  
 Sbjct: 563 IAARERLDIGAREIENREAALLSSSGDLHIGSALNGSRQVQGANTSLHNRSAAISS--- 619  
 Query: 626 GNI 628  
 GNI  
 Sbjct: 620 GNI 622  
  
 Score = 37.5 bits (85), Expect = 0.53  
 Identities = 87/432 (20%), Positives = 159/432 (36%), Gaps = 62/432 (14%)  
 Query: 239 LQGLQGKNLAVSTGPKVDYASGEISAGTAAGTKPTIALDTAALGMYADSITLIAXEK 298  
 LQG LQGKN+ + G + +G I A A K A + + S T +  
 Sbjct: 1023 LQGLQGKNIFAAAGSDITN--TGSIGAENALLK-----ASNIESRSETRSNQNE 1072  
 Query: 299 GVGKVNAGTLEAAKQLIVTSSGRI--ENSGRIATTADGTEASPTYLXIETTEKGAXG-TF 355  
 V+N G + A L +G + + I TA E T + G T  
 Sbjct: 1073 QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTAS-----ELTNQSEDGQTV 1120  
 Query: 356 ISNGGRIESKGLLVIIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESK-----T 408  
 ++ GG I S + I + V++ + +T+ G NL + +K  
 Sbjct: 1121 LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIRAA 1179  
 Query: 409 NVNNAKGSXNLSAGGRITINDATIQAGSS-----VYSSTKGD TXLGENTRIIAENV 460  
 V + +G L+AG D ++AG + Y+ G + TR +  
 Sbjct: 1180 EVGSEQRLKLAAG-----RDIKVEAGKAHTETEDALKYTGRSGGGIKQKMRHLKNQNG 1234  
 Query: 461 VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASNIRLNNGNIKGGKQLALLADDNIT 520  
 +G++ +I +G + + T+ S NN +K + + A+ N  
 Sbjct: 1235 QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILS--AKNNIVLKAETRSRSAEMNKK 1292  
 Query: 521 AKTTNLNTPG-NLYVHTGKDLNLNVDKDLASAASIHLSKDN-----AAHITGTSKTLTA 572  
 K+ + + G + KD N + +S + S N H T T T+++  
 Sbjct: 1293 EKSGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISS 1352  
 Query: 573 SK-DMGVEAGXXXXXXXXXXXXSGNLHIQAAGK-----NIQLRNTKLNAAKALETALQG 626  
 + D+G+ +G + + KG ++ + NT + A A++ G  
 Sbjct: 1353 PQGDVGISSGKISIDAAQNRYSQESKQVYEQKGVTVVAISVPVVNTVMGAVDAVKAVQTVG 1412  
 Query: 627 NIVSDGLHAVSA 638  
 + ++A++A  
 Sbjct: 1413 KSKNSRVNMAAA 1424

Amino acids 1-1423 of ORF114-1 were cloned in the pGex vector and expressed in *E. coli*, as described above. GST-fusion expression was visible using SDS-PAGE, and Figure 5 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF114-1.

Based on these results, including the homology with the putative secreted protein of *N. meningitidis* and on the presence of a transmembrane domain, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

#### Example 14

The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 63>



-95-

```

1  ..CGCTTCATTC ATGATGAAGC AGTCGGCAGC AACATCGGCG GCGGCAAAAT
51  GATTGTTGCA GCCGGGCAGG ATATCAATGT ACGCGGCAnA AGCCTTATTT
101 CTGATAAGGG CATTTGTTTTA AAAGCAGGAC ACGACATCGA TATTTCTACT
151 GCCCATAATC GCTATACCGG CAATGAATAC CACGAGAGCA wAAAwTCAGG
5  201 CGTCATGGGT ACTGGCGGAT TGGGCTTTAC TATCGGTAAC CGGAAAACATA
251 CCGATGACAC TGATCGTACC AATATTGTsC ATACAGGCAG CATTATAGGC
301 AGCGTGAaTG GAGACACCGT TACAGTTGCA GGAaACCGCT ACCGACAAAC
351 CGGCAGTACC GTCTCCAGCC CCGAGGGGCG CAATACCGTC ACAGCCAAAw
10  401 GCATAGATGT AGAGTTCGCA AACAAACCGGT ATGCCACTGA CTACGcCCAT
451 ACCCAgGGAA CAAAAAGGCC TTACCGTCGC CCTCAATGTC CCGGTTGTCC
501 AAGCTGCACA AAACCTTCATA CAAGCAGCCC AAAATGTGGG CAAAAGTAAA
551 AATAAACGCG TTAATGCCAT GGCTGCAGCC AATGCTGCAT GGCAGAGTTA
601 TCAAGCAACC CAACAAATGC AACAAATTGC TCCAAGCAGC AGTGGCGGAC
15  651 AAGGTCAAAA CTACAATCAA AGCCCCAGTA TCAGTGTGTC CATTAC.TAC
701 GGCgAACAGA AAAGTCGTAA CGAGCAAAA AGACATTACA CCGAAgCGGC
751 AgCAAGTCAA ATTATCGGCA AAGGGCAAA CACACTTGCG GCAACAGGAA
801 GTGGGGAGCA GTCCAATATC AATATTACAG GTTCCGATGT CATCGGCCAT
851 GCAGGTACTC C.CTCATTGC CGACAACCAT ATCAGACTCC AATCTGCCAA
20  901 ACAGGACGGC AGCGAGCAA GCAAAAACAA AAGCAGTGGT TGGAAATGCAG
951 GCGTACGTnn CAAATAGGC AACGGCATCA GGT'TTGGAAAT TACCGCCGGA
1001 GGAAATATCG GTAAAGGTAA AGAGCAAGGG GGAAGTACTA CCCACCGCCA
1051 CACCCATGTC GGCAGCACAA CCGGCAAAAC TACCATCCGA AGCGCGGGGg
1101 GATACCACCC TCAAAGGTGT GCAGCTCATC GGCAAAGGCA TACAGGCAGa
1151 TACGCGCAAC CTGCATATAG AAAGTGTTCA AGATACTGAA ACCTATCAGA
25  1201 GCAAAACAGCA AAACGGCAAT GTCCAAGTTt ACTGTCGGTT ACGGATTcAG
1251 TCGAAGCGGC AGTTACCGCC AAAGCAAAGT CAAAGCAGAC CATGCTCCG
1301 TAACCGGGCA AAgCGGTATT TATGCCGGAG AAGACGGCTA TCAAATyAAA
1351 GTyAGAGACA ACACAGACCT yAAGGGCGGT ATCATCACGT CTAGCCAAAG
1401 CGCAGAAGAT AAGGGCAAAA ACCTTTTTCA GACGGCCACC CTTACTGCCA
30  1451 GCGACATTCA AAACACAGC CGCTACGAAG GCAGAAGCTT CGGCATAGGC
1501 GGCAGTTTCG ACCTGAACGG CGGCTGGGAC GGCACGGTTA CCGACAAACA
1551 AGGCAGGCCT ACCGACAGGA TAAGCCCGGC AGCCGGCTAC GGCAGCGACG
1601 GAGACAGCAA AAACAGCACC ACCCGCAGCG GCGTCAACAC CCACAACATA
1651 CACATCACCG ACGAAGCGGG ACAACTTGCC CGAACAGGCA GGACTGCAAA
35  1701 AGAAACCGAA GCGCGTATCT ACACCGGCAT CGACACCGAA ACTGCGGATC
1751 AACACTCAGG CCATCTGAAA AACAGCTTCG AC...

```

This corresponds to the amino acid sequence <SEQ ID 64; ORF116>:

```

1  ..RFIHDEAVGS NIGGGKMIVA AGQDINVRGX SLISDKGIVL KAGHDIDIST
40  51  AHNRYTGNEY HESXXSGVMG TGGLGFTIGN RKTTDDTDRT NIVHTGSIIG
101  SLNGDVTVTA GNRYRQTGST VSSPEGRNTV TAKXIDVEFA NNRYATDYAH
151  TQEQKGLTVA LNVPVVQAAQ NFIQAAQNVG KSKNKRNVAM AAANAAWQSY
201  QATQMQQFPA PSSSAGQGQN YNQSPSISVS IXYPEQKSRN EQKRHYTEAA
251  ASQIIKGQT TLAAATGSEQ SNINITGSDV IGHAGTXLIA DNHIRLSAK
45  301  QDGSEQSKNK SSGWNAGVRX KIGNGIRFGI TAGGNIGK GK EQGGSTTHRH
351  THVGSTTGKT TIRSGDRTL KGVQLIGKI QADTRNLHIE SVQDTETYQS
401  KQQNGNVQVT VGYGFSASGS YRQSKVKADH ASVTGQSGIY AGEDGYQIKV
451  RDNTDLKGGI ITSSQSAEDK GKNLFQTATL TASDIQNH SR YEGRSFGIGG
501  SFDLNGGWDG TVTDKQGRPT DRISPAAGYG SDGDSKNSTT RSGVNTHNIH
551  ITDEAGQLAR TGR TAKETEA RIYTGIDTET ADQHSGLHKN SFD...

```

50 Computer analysis of this amino acid sequence gave the following results:

Homology with pspA putative secreted protein of *N.meningitidis* (accession number AF030941)

ORF116 and pspA protein show 38% aa identity in 502aa overlap:

```

Orf116: 6  EAVGSNIGGGKMIVAAGQDINVRGXSLISDKGIVLKAGHDIDISTAHNRYTGNEYHESXX 65
55  +AV + G ++I+ +G+DI V G ++I+D +L A ++I + A R E ++
PspA: 1 235 QAVSGTLDGKEIILVSGRDITVTGSNIADNHTILSAKNNIVLKAETRSRSAEMNKKEK 1294
Orf116: 66  XXXXXXXXXXXXXXXNRXXXXXXXXRTNIVHTGSIIGSLNGDVTVAGNRYRQTGSTVSSPE 125
++K + HT S++GSLNG+T+ AG Y QTGST+SSP+
PspA: 1295 SGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISSPQ 1354
60

```

Orf116: 126 GRNTVTAKXIDVEFANNRYATDYAHTQEQKGLTVALNVPXXXX--XXXXXXXXXXXXXGKS 182  
 G +++ I ++ A NRY+ + EQKG+TVA++VP GKS  
 PspA: 1355 GDVGISSGKISIDAAQNRYSESQKQVYEQKGVTVVAISVPVNTVMGAVDKAVQTVGKS 1414

5 Orf116: 183 KNKRXXXXXXXWQSYQATQMQQFA--PSSSAGQGQNYNQSPSISVSIXYGEQKSRN 240  
 KN RV + + + A P +AGQG ISVS+ YGEQK+ +  
 PspA: 1415 KNSRVNMAAANALNKGVDSGVALYNAARNPKKAAGQG-----ISVSVTYGEQKNTS 1466

10 Orf116: 241 EQKRHYTEAAASQIIGKGQTTLAATGSGEQSNINITGSDVIGHAGTXLIADNHIRLQSAK 300  
 E + T+ +I G G+ +L A+G+G+ S I ITGSDV G GT L A+N ++++A+  
 PspA: 1467 ESRIKGTQVQEGKITGGGKVSILTASGAGKDSRITITGSDVYGGKGTRLKAENAVQIEAAR 1526

15 Orf116: 301 QDGSEQSKNKSSGWNAGVRXKIGNGIRFGITAXXXXXXXXXXXXXSTTHRHTHVGSTTGKT 360  
 Q E+S+NKS+G+NAGV I GI FG TA T +R++H+GS +T  
 PspA: 1527 QTHQERSENKSAGFNAGVAIAINKGISFGFTAGANYGKGYNGDETAYRNSHIGSKDSQT 1586

20 Orf116: 361 TIRSGGDTTLKGVQLIGKGIQADTRNLHIESVQDTETYQSKQONGNVQTVGYGFSASGS 420  
 I SGGDT +KG QL GKG+ +LHIES+QDT ++ KQ+N + QVTVGYGFS GS  
 PspA: 1587 AIESGGDTVIKGGQLKGGKGVGTAEHLHIESLQDTAVFKGQENVSAQVTVGYGFSVGGGS 1646

25 Orf116: 421 YRQSKVKADHASVTGQSGIYAGEDGYQIKVRDNTDLKGGIITSSQSAEDKGNLFQTATL 480  
 Y +SK +D+ASV QSGI+AG DGY+I+V T L G + S DK KNL +T+ +  
 PspA: 1647 YNRKSSSDYASVNEQSGIFAGGDGYRIRVNGKTGLVGAADVSD---ADKSKNLLKTSEI 1703

Orf116: 481 TASDIQNHSRYEGRSFGIGGSF 502  
 DIQNH+ + G+ G F  
 PspA: 1704 WHKDIQNHASAAASALGLSGGF 1725

Based on homology with *pspA*, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

### 30 Example 15

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 65>

35 1 ..ACGACCGGCA GCCTCGGCGG CATACTGGCC GCGCGCGGCA CTTCCCTTGC  
 51 CGCACCGTAT TTGGACAAAG CGGCGGAAAA CCTCGGTCCG GCGGGCAAAG  
 101 CCGCGGTCAA CGCACTGGGC GGTGCGGCCA TCGGCTATGC AACTGGTGGT  
 151 AGTGGTGGTG CTGTGGTGGG TGCGAATGTA GATTGGAACA ATAGGCAGCT  
 201 GCATCCGAAA GAAATGGCGT TGGCCGACAA ATATGCCGAA GCCCTCAAGC  
 251 GCGAAGTTGA AAAACGCGAA GGCAGAAAAA TCAGCAGCCA AGAAGCGGCA  
 301 ATGAGAATCC GCAGGCAGAT ATGCGTTGGG TGGACAAAGG TTCCCAAGAC  
 351 GGCTATACCG ACCAAAGCGT CATATCCCTT ATCGGAATGA

40 This corresponds to the amino acid sequence <SEQ ID 66; ORF118>:

1 ..TTGSLGGILA GGGTSLAAPY LDKAAENLGP AGKAAVNALG GAAIGYATGG  
 51 SGGAVVGANV DWNRRQLHPK EMALADKYAE ALKREVEKRE GRKISSQEAA  
 101 MRIRRQICVG WTKVPKTAIP TKASYPLSE\*

Computer analysis of this amino acid sequence reveals two putative transmembrane domains.

45 Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

### Example 16

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 67>

1 ..CAATGCCGTC TGAAAAGCTC ACAATTTTAC AGACGGCATT TGTTATGCAA

51 GTACATATAC AGATTCCCTA TATACTGCCC AGrkGCGTGC GTgGCTGAAG  
 101 ACACCCCCTA CGCTTGCTAT TTGrAACAGC TCCAAGTCAC CAAAGACGTC  
 151 AACTGGAACC AGGTACwACT GCGGTACGAC AAATGGGACT ATAAACAGGA  
 201 AGGCTTAACC GGAGCCGGAG CAGCGATTAT TCGCTGGCT GTTACCGTGG  
 5 251 TTA CTGCGGG CGCGGGAgCC GGAGCCGCAC TGGGcTTAA CCGCGCGGcC  
 301 GCAGCGGCAA CCGATGCCGC ATTGCGCTCG CTGGCCAGCC AGGcTTCCGT  
 351 ATCGCTCATC AaCAACAAAG GCAATATCGG TAaCACCCTG AAAGAGCTGG  
 401 GCAGAAGCAG CACGGTGAAA AATCTGATGG TTGCCGTCGc tACCGCAgGC  
 10 451 GTagCcgaCA AAATCGGTGC TTCGGCACTG AACAATGTCA CGCGATAAGCA  
 501 GTGGATCAAC AACCTGACCG TCAACCTGGC CAATGCGGGC AGTGCCGCAC  
 551 TGATTAATAC CGCTGTCAAC GGCGGCAGCc tgAAAGACAA TCTGGAAGCG  
 601 AATATCCTTG CCGCTTTGGT GAATACTGCG CATGGAGAAG CAGCCAGTAA  
 651 AATCAAAACAG TTGGATCAGC ACTACATTAC CCACAAGATT GCCcATGCCA  
 15 701 TAGCGGGCTG TCGGcTGCG GCGGCGAATA AGGGCAAGTG TCAGGATGGT  
 751 GCGATAgGTG CGGCTGTGGG CGAGATAGTC GGGGAgGCTT TGACAAACGG  
 801 CAAAAATCCT GACACTTTGA CAGCTAAAgA ACGCGaACAG ATTTTGGCAT  
 851 ACAGCAAACCT GGTGCGCGGT ACGGTAAGCG GTGTGGTTCG CCGCGATGTA  
 901 AATGCGGCGG CGAATGCGGC TGAGGTAGCG GTGAAAAATA ATCAGCTTAG  
 951 CGACAAAtGA

20 This corresponds to the amino acid sequence <SEQ ID 68; ORF41>:

1 ..QCRLKSSQFY RRHLLCKYIY RFPIYCPXAC VAEDTPYACY LXQLQVTKDV  
 51 NWNQVXLAYD KWDYKQEGLT GAGAAI IALA VTVVTAGAGA GAALGLNGAA  
 101 AAATDAAFAS LASQASVSLI NKNKNIGNTL KELGRSSTVK NLMVAVATAG  
 151 VADKIGASAL NNVSDKQWIN NLTVNLANAG SAALINTAVN GGSLLKDNLEA  
 25 201 NILAALVNTA HGEAASKIKQ LDQHYITHKI AHAIAGCAA AANKGKCQDG  
 251 AIGAAVGEIV GEALTNGKNP DLTAKEREQ ILAYSKLVAG TVSGVVGGDV  
 301 NAAANAAEVA VKNNQLSDK\*

Further work revealed the complete nucleotide sequence <SEQ ID 69>:

1 ATGCAAGTAA ATATTCAGAT TCCCTATATA CTGCCCAGAT GCGTGCGTGC  
 30 51 TGAAGACACC CCCTACGCTT GCTATTTGAA ACAGCTCCAA GTCACCAAAG  
 101 ACGTCAACTG GAACCAAGTA CAACTGGCGT ACGACAAATG GGACTATAAA  
 151 CAGGAAGGCT TAACCGGAGC CGGAGCAGCG ATTATTGCGC TGGCTGTTAC  
 201 CGTGTTACT GCGGGCGCGG GAGCCGGAGC CGCACTGGGC TTAAACGGCG  
 251 CCGCGCAGC GGCAACCGAT GCCGCATTG CCGCTGCTGGC CAGCCAGGCT  
 35 301 TCCGTATCGC TCATCAACAA CAAAGGCAAT ATCGGTAACA CCCTGAAAGA  
 351 GCTGGGCAGA AGCAGCACGG TGAAAAATCT GATGGTTGCC GTCGCTACCG  
 401 CAGGCGTAGC CGACAAAATC GGTGCTTCGG CACTGAACAA TGTGACGCGAT  
 451 AAGCAGTGGA TCAACAACCT GACCGTCAAC CTGGCCAATG CGGGCAGTGC  
 501 CGCACTGATT AATACCGCTG TCAACGCGCG CAGCCTGAAA GACAATCTGG  
 40 551 AAGCGAATAT CTTGCGGCT TTGGTGAATA CTGCGCATGG AGAAGCAGCC  
 601 AGTAAATCA AACAGTTGGA TCAGCACTAC ATTACCCACA AGATTGCCCA  
 651 TGCCATAGCG GGCTGTGCGG CTGCGGCGGC GAATAAGGGC AAGTGTGAGG  
 701 ATGGTGCGAT AGGTGCGGCT GTGGGCGAGA TAGTCGGGGA GGCTTTGACA  
 751 AACGGCAAAA ATCCTGACAC TTTGACAGCT AAAGAACGCG AACAGATTTT  
 45 801 GGCATACAGC AAACCTGGTG CCGGTACGGT AAGCGGTGTG GTCGCGGGCG  
 851 ATGTAAATGC GGCGGCGAAT GCGGCTGAGG TAGCGGTGAA AAATAATCAG  
 901 CTTAGCGACA AAGAGGGTAG AGAATTTGAT AACGAAATGA CTGCATGCGC  
 951 CAAACAGAAT AATCCTCAAC TGTGCAGAAA AAATACTGTA AAAAAGTATC  
 1001 AAAATGTTGC TGATAAAAGA CTTGCTGCTT CGATTGCAAT ATGTACGGAT  
 50 1051 ATATCCCGTA GTACTGAATG TAGAACAATC AGAAAACAAC ATTTGATCGA  
 1101 TAGTAGAAGC CTTCAATTCAT CTTGGGAAGC AGGTCTAATT GGTAAAGATG  
 1151 ATGAATGGTA TAAATTATTC AGCAAATCTT ACACCCAAGC AGATTTGGCT  
 1201 TTACAGTCTT ATCATTGAA TACTGCTGCT AAATCTTGGC TTCAATCGGG  
 1251 CAATACAAAG CTTTATCCG AATGGATGTC CGACCAAGGT TATACACTTA  
 55 1301 TTTAGGAGT TAATCCTAGA TTCATTCCAA TACCAAGAGG GTTTGTAAAA  
 1351 CAAAATACAC CTATTACTAA TGTCAAATAC CCGGAAGGCA TCAGTTTCGA  
 1401 TACAAACCTA AAAAGACATC TGGCAAATGC TGATGGTTTT AGTCAAAAC  
 1451 AGGGCATTA AGGAGCCCAT AACCACACCA ATTTTATGGC AGAACTAAAT  
 1501 TCACGAGGAG GACGCGTAAA ATCTGAAACC CAACTGATA TTGAAGGCAT  
 60 1551 TACCCGAATT AATATTGAGA TTCTACACT AGACAGGACA GGTAACCTG  
 1601 ATGGTGGATT TAAGGAAATT TCAAGTATAA AAATGTTTA TAATCCTAAA  
 1651 AAATTTCTG ATGATAAAAT ACTTCAAATG GCTCAAATG CTGCTTCACA  
 1701 AGGATATTCA AAAGCCTCTA AAATTGCTCA AAATGAAAGA ACTAAATCAA  
 1751 TATCGGAAAG AAAAAATGTC ATTCAATTCT CAGAAACCTT TGACGGAATC  
 65 1801 AAATTTAGAT CATATTTTGA TGTAATATACA GGAAGAATTA CAAACATTCA  
 1851 CCCAGAATAA

This corresponds to the amino acid sequence <SEQ ID 70; ORF41-1>:

```

      1  MQVNIQIPYI  LPRCVRAEDT  PYACYLKQLQ  VTKDVNWNQV  QLAYDKWDYK
    51  QEGLTGAGAA  IIALAVTVVT  AGAGAGAALG  LNGAAAAATD  AAFASLASQA
   101  SVSLINNKGK  IGNTLKELGR  SSTVKNLMVA  VATAGVADKI  GASALNNVSD
    151  KQWINNLTVN  LANAGSAAAI  NTAVNGGSLK  DNLEANILAA  LVNTAHGEAA
    201  SKIKQLDQHY  ITHKIAHAIA  GCAAAAANKG  KCQDGAIGAA  VGEIVGEALT
    251  NGKNPDTLTA  KEREQILAYS  KLVAGTVSGV  VGGDVNAAAN  AAEVAVKNNQ
    301  LSDKEGREFD  NEMTACAKQN  NPQLCRKNTV  KKYQNVADKR  LAASIAICTD
    351  ISRSTECRTI  RKQHLIDSRS  LHSSWEAGLI  GKDDEWYKLF  SKSYTQADLA
    401  LQSYHLNTAA  KSWLQSGNTK  PLSEWMSDQG  YTLISGVNPR  FIPIPRGFVK
    451  QNTPITNVKY  PEGISFDTNL  KRHLANADGF  SQKQGIKGAH  NRTNFMALN
    501  SRGGRVKSET  QTDIEGITRI  KYEIPDLDR  GKPDGGFKEI  SSIKTVYNPK
    551  KFSDDKILQM  AQNAASQGY  KASKIAQNER  TKSISERKNV  IQFSETFDGI
    601  KFRSYFDVNT  GRITNIHPE*

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15 Computer analysis of this amino acid sequence predicts a transmembrane domain, and homology with an ORF from *N.meningitidis* (strain A) was also found.

ORF41 shows 92.8% identity over a 279aa overlap with an ORF (ORF41a) from strain A of *N. meningitidis*:

```

20  orf41.pep  10      20      30      40      50      60      69
    YRRHLLCKYIYRFPIYCPXACVAEDTPYACYLXQLQVTKDVNWNQVXLAYDKWDYKQEGGL
    orf41a      || ||||:|:||||| ||||:|||||
                  YLKQLQVAKNINWNQVQLAYDRWDYKQEGGL
                        10      20      30

25  orf41.pep  70      80      90      100     110     120     129
    TGAGAAIIALAVTVVTAGAGAGAALGLNGAAAAATDAAFASLASQASVSLINNKGNI
    orf41a      | |||||:||||:||||:||||| |||||:||||:||||:|
    TEAGAAIIALAVTVVTSGAGTGAVLGLNGAXAAATDAAFASLASQASVFINNKG
                        40      50      60      70      80      90

30  orf41.pep  130     140     150     160     170     180     189
    LKELGRSSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLTVNLANAGSAA
    orf41a      |||||:||||:||||:||||| |||||:||||:||||:|
    LKELGRSSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLTVNLANAGSAA
                        100     110     120     130     140     150

35  orf41.pep  190     200     210     220     230     240     249
    NGGSLKDNLEANILAALVNTAHGEAAASKIKQLDQHYITHKIAHAIAAGCAAAA
    orf41a      |||||:||||:||||:||||| |||||:||||:||||:|
    NGGSLKDXLEANILAALVNTAHGEAAASKIKQLDQHYIVHKIAHAIAAGCAAAA
                        160     170     180     190     200     210

40  orf41.pep  250     260     270     280     290     300     309
    GAIGA AVGEIVGEALTNGKNPDTLTAKEREQILAYS KLVAGTVSGV VGGDVN
    orf41a      |||||:||||:||||:||||| |||||:||||:||||:|
    GAIGA AVGEIVGEALTNGKNPDTLTAKEREQILAYS KLVAGTVSGV VGGDVN
                        220     230     240     250     260     270

45  orf41.pep  310     320
    AVKNNQLSDKX
    orf41a      |||||
    AVKNNQLSDXEGREFDNEMTACAKQNPQLCRKNTVKKYQNVADKRLAASIAICTDISRS
                        280     290     300     310     320     330

```

A partial ORF41a nucleotide sequence <SEQ ID 71> is:

```

55  1  ..TATCTGAAAC AGCTCCAAGT AGCGAAAAAC ATCAACTGGA ATCAGGTGCA
    51  GCTTGCTTAC GACAGATGGG ACTACAAACA GGAGGGCTTA ACCGAAGCAG
   101  GTGCGGCGAT TATCGCACTG GCCGTTACCG TGGTCACCTC AGGCGCAGGA
   151  ACCGGAGCCG TATTGGGATT AAACGGTGCG NCCGCCGCCG CAACCGATGC

```

201 AGCATTCGCC TCTTTGGCCA GCCAGGCTTC CGTATCGTTC ATCAACAACA  
 251 AAGGCGATGT CGGCAAAACC CTGAAAGAGC TGGGCAGAAG CAGCACGGTG  
 301 AAAAATCTGG TGGTTGCCGC CGCTACCGCA GGCCTAGCCG ACAAATCGG  
 351 CGCTTCGGCA CTGANCAATG TCAGCGATAA GCAGTGGATC AACAACTGA  
 401 CCGTCAACCT AGCCAATGCG GGCAGTGCCG CACTGATTAA TACCGTGTG  
 451 AACGGCGGCA GCCTGAAAGA CANTCTGGAA GCGAATATCC TTGCGGCTTT  
 501 GGTCAATACC GCGCATGGAG AAGCAGCCAG TAAAATCAAA CAGTTGGATC  
 551 AGCACTACAT AGTCCACAAG ATTGCCCATG CCATAGCGGG CTGTGCGGCA  
 601 GCGGCGGCGA ATAAGGGCAA GTGTCAGGAT GGTGCGATAG GTGCGGTGT  
 651 GGGCGAGATA GTCGGGGAGG CTTTGACAAA CGGCAAAAAT CCTGACACTT  
 701 TGACAGCTAA AGAACGCGAA CAGATTTTGG CATACAGCAA ACTGGTTGCC  
 751 GGTACGGTAA GCGGTGTGGT CCGCGGCGAT GTAAATGCGG CGGCGAATGC  
 801 GCGTGAAGTA GCGGTGAAA ATAATCAGCT TAGCGACNAA GAGGTAGAG  
 851 AATTTGATAA CGAAATGACT GCATGCGCCA AACAGAATAN TCCTCAACTG  
 901 TGCAGAAAAA ATACTGTAAA AAAGTATCAA AATGTTGCTG ATAAAAGACT  
 951 TGCTGCTTCG ATTGCAATAT GTACGGATAT ATCCCGTAGT ACTGAATGTA  
 1001 GAACAATCAG AAAACAACAT TTGATCGATA GTAGAAGCCT TCATCTACT  
 1051 TGGGAAGCAG GTCTAATTGG TAAAGATGAT GAATGGTATA AATTATTGAG  
 1101 CAAATCTTAC ACCCAAGCAG ATTTGGCTTT ACAGTCTTAT CATTGGAATA  
 1151 CTGCTGCTAA ATCTTGGCTT CAATCGGGCA ATACAAAGCC TTTATCCGAA  
 1201 TGGATGTCG ACCAAGGTTA TACACTTATT TCAGGAGTTA ATCCTAGATT  
 1251 CATTCGAATA CCAAGAGGGT TTGTAAAACA AAATACACCT ATTACTAATG  
 1301 TCAAATACCC GGAAGGCATC AGTTTCGATA CAAACCTANA AAGACATCTG  
 1351 GCAAATGCTG ATGGTTTTAG TCAAGAACAG GGCATTAAAG GAGCCCATAA  
 1401 CCGCACCAAT NTTATGGCAG AACTAAATTC ACGAGGAGGA NGNGTAAAT  
 1451 CTGAAACCCA NACTGATATT GAAGGCATTA CCCGAATTAA ATATGAGATT  
 1501 CCTACACTAG ACAGGACAGG TAAACCTGAT GGTGGATTTA AGGAAATTC  
 1551 AAGTATAAAA ACTGTTTATA ATCCTAAAAA NTTTTNNGAT GATAAAATAC  
 1601 TTCAAATGGC TCAANATGCT GNTTCACAAG GATATTCAAA AGCCTCTAAA  
 1651 ATTGCTCAAA ATGAAAGAAC TAAATCAATA TCGGAAAGAA AAAATGTCAT  
 1701 TCAATTCTCA GAAACCTTTG ACGGAATCAA ATTTAGANNN TATNTNGATG  
 1751 TAAATACAGG AAGAATTACA AACATTCACC CAGAATAA

This encodes a protein having the partial amino acid sequence <SEQ ID 72>:

1 YLKQLQVAKN INWNQVQLAY DRWDYKQEGL TEAGAAIAL AVTVVTSAG  
 51 TGAVLGLNGA XAAATDAFA SLASQASVSF INNKGVDGKT LKELGRSSTV  
 101 KNLVVAATA GVADKIGASA LXNVSDKQWI NNLTVNLANA GSAALINTAV  
 151 NGGSLKDXLE ANILAALVNT AHGEAASKIK QLDQHYIVHK IAHAIAGCAA  
 201 AAANKGKCQD GAIGAANVEI VGEALTNGKN PDTLTAKERE QILAYSKLVA  
 251 GTVSGVVGDD VNAAANAEV AVKNNQLSDX EGREFDNEMT ACAKONXPQL  
 301 CRKNTVKKYQ NVADKRLAAS IAICTDISRS TECRTIRKQH LIDSRSLHSS  
 351 WEAGLIGKDD EWYKLFSKSY TQADLALQSY HLNATAKSWL QSGNTPKPLSE  
 401 WMSDQGYTLI SGVNPRFIPI PRGFVKQNTF ITNVKYPEGI SFDNLXRLH  
 451 ANADGFSQEQ GIKGAHNRTN XMAELNSRGG XVKSETXTDI EGITRIKYEI  
 501 PTLDRGTGKPD GGFKEISSIK TVYNPKXFXD DKILQMAQXA XSQGYSKASK  
 551 IAQNERTKSI SERKNVIQFS ETFDGIKFRX YXDVTGTRIT NIHPE\*

ORF41a and ORF41-1 show 94.8% identity in 595 aa overlap:

orf41a.pep YLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAA  
 50 orf41-1 MQVNIQIPYILPRCVRAEDTPYACYLKQLQVTKDVNWNQVQLAYDKWDYKQEGLTGAGAA  
 10 20 30 40 50 60  
 orf41a.pep IIALAVTVVTSAGTGAVLGLNGAXAAATDAAFASLASQASVSFINNKGVDVGKTLKELGR  
 55 orf41-1 IIALAVTVVTAGAGAGAALGLNGAAAAATDAAFASLASQASVSLINNKNIGNTLKELGR  
 70 80 90 100 110 120  
 orf41a.pep SSTVKNLVVAATAGVADKIGASALXNVSDKQWNNLTVNLANAGSAALINTAVNGGSLK  
 60 orf41-1 SSTVKNLMVAVATAGVADKIGASALNNVSDKQWNNLTVNLANAGSAALINTAVNGGSLK  
 130 140 150 160 170 180  
 orf41a.pep DXLEANILAALVNTAHGEAASKIKQLDQHYIVHKIAHAIAGCAAAAANKGKCQDGAIGAA  
 65

-100-

	orf41-1	DNLEANILAAALVNTAHGEAASKIKQLDQHYITHKIAHAIAGCAAAAANKGKCQDGAIGAA
		190 200 210 220 230 240
5	orf41a.pep	VGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAAANAEEVAVKNNQ
	orf41-1	VGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAAANAEEVAVKNNQ
10		250 260 270 280 290 300
	orf41a.pep	LSDXEGREFDNEMTACAKQNPQLCRKNTVKKYQNVADKRLAASIAICTDISRSTECRTI
15	orf41-1	LSDXEGREFDNEMTACAKQNPQLCRKNTVKKYQNVADKRLAASIAICTDISRSTECRTI
		310 320 330 340 350 360
	orf41a.pep	RKQHLIDSRSLHSSWEAGLIGKDDWEYKLFSSYQADLALQSYHLNTAAKSWLQSGNTK
20	orf41-1	RKQHLIDSRSLHSSWEAGLIGKDDWEYKLFSSYQADLALQSYHLNTAAKSWLQSGNTK
		370 380 390 400 410 420
	orf41a.pep	PLSEWMSDQGYTLISGVNPRFIPIPRGFVKQNTPTITNVKYPEGISFDTNLXRHLANADGF
25	orf41-1	PLSEWMSDQGYTLISGVNPRFIPIPRGFVKQNTPTITNVKYPEGISFDTNLKRHLANADGF
		430 440 450 460 470 480
	orf41a.pep	SQEQGIKGAHNRTNXMAELNSRGGXVKSETXTDIEGITRIKYEIPTLDRTGKPDGGFKEI
30	orf41-1	SQEQGIKGAHNRTNFMELNSRGGXVKSETXTDIEGITRIKYEIPTLDRTGKPDGGFKEI
		490 500 510 520 530 540
	orf41a.pep	SSIKTVYNPKXFXDDKILQMAQXASQGYSKASKIAQNERTKSI SERKNVIQFSETFDGI
35	orf41-1	SSIKTVYNPKXFXDDKILQMAQNAASQGYSKASKIAQNERTKSI SERKNVIQFSETFDGI
		550 560 570 580 590 600
40	orf41a.pep	KFRXYXDVNTGRITNIHPEX
	orf41-1	KFRSYFDVNTGRITNIHPEX
45		610 620

Amino acids 25-619 of ORF41-1 were amplified as described above. Figure 6 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF41-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

## 50 Example 17

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 73>

	1	ATGGCAATCA	TTACATTGTA	TTATTCTGTC	AATGGTATTT	TAAATGTATG
	51	TGCAAAAGCA	AAAAATATTC	AAGTAGTTGC	CAATAATAAG	AATATGGTTC
55	101	TTTTTGGGTT	TTTGGsmrGC	ATCATCGGCG	GTTCAACCAA	TGCCATGTCT
	151	CCCATATTGT	TAATATTTTT	GCTTAGCGAA	ACAGAAAATA	AAAATcgTAT
	201	CGTAAAATCA	AGCAATCTAT	GCTATCTTTT	GGCGAAAATT	GTTCAAATAT
	251	ATATGCTAAG	AGACCAATAT	TGGTTATTAA	ATAAGAGTGA	ATACGdTTTA
	301	ATATTTTTAC	TGTCCGTATT	GTCTGTTATT	GGATTGTATG	TTGGAATTCG
	351	GTTAAGGACT	AAGATTAGCC	CAaATTTTTT	TAAAATGTTA	ATTTTTATTG

401 tTTTATTGGT ATTGGCtCTG AAAATCGGGC AttCGGGTTT AAtCAAACCTT  
451 TAA

This corresponds to the amino acid sequence <SEQ ID 74; ORF51>:

5           1   MAIITLYYSV NGILNVCACA KNIQVVANNK NMVLFGLXX IIGGSTNAMS  
          51   PILLIFLLSE TENKNRIVKS SNLCYLLAKI VQIYMLRDQY WLLNKSEYXL  
         101   IFLLSVLSVI GLYVGIRLRT KISPNEFKML IFIVLLVLAL KIGHSGLIKL  
         151   \*

Further work revealed the complete nucleotide sequence <SEQ ID 75>:

10           1   ATGCAAGAAA TAATGCAATC TATCGTTTTT GTTGCTGCCG CAATACTGCA  
          51   CGGAATTACA GGCATGGGAT TTCCGATGCT CGGTACAACC GCATTGGCCT  
         101   TTATCATGCC ATTGTCTAAG GTTGTTCCT TGGTGGCATT ACCAAGCCTG  
         151   TTAATGAGCT TGTGGTTCT ATGCAGCAAT AACAAAAAGG GTTTTTGGCA  
         201   AGAGATTGTT TATTATTTAA AAACCTATAA ATTGCTTGCT ATCGGCAGCG  
         251   TCGTTGGCAG CATTTTGGGG GTGAAGTTGC TTTTGATACT TCCAGTGTCT  
15           301   TGGCTGCTTT TACTGATGGC AATCATTACA TTGTATTATT CTGTCAATGG  
         351   TATTTTAAAT GTATGTGCAA AAGCAAAAAA TATTCAAGTA GTTGCCAATA  
         401   ATAAGAATAT GGTTCCTTTT GGGTTTTTGG CAGGCATCAT CGGCGGTTCA  
         451   ACCAATGCCA TGTCTCCCAT ATTGTTAATA TTTTGTCTTA GCGAAACAGA  
         501   AAATAAAAAT CGTATCGTAA AATCAAGCAA TCTATGCTAT CTTTTGGCGA  
20           551   AAATTGTTCA AATATATATG CTAAGAGACC AGTATTGGTT ATTAAATAAG  
         601   AGTGAATACG GTTTAATATT TTTACTGTCC GTATTGTCTG TTATTGGATT  
         651   GTATGTTGGA ATTCGGTTAA GGAATAAGAT TAGCCCAAAT TTTTTTAAAA  
         701   TGTTAATTTT TATTGTTTTA TTGGTATTGG CTCTGAAAAT CGGGCATTCTG  
         751   GGTTTAATCA AACTTTAA

25   This corresponds to the amino acid sequence <SEQ ID 76; ORF51-1>:

          1   MQEIMQSIVF VAAAILHGIT GMGFPLGTT ALAFIMPLSK VVALVALPSL  
         51   LMSLLVLCNS NKKGFWEIV YYLKYKLLA IGSVVSILG VKLLLILPVS  
         101   WLLLLMAIIT LYYSVNGILN VCAKAKNIQV VANNKNMVLG GFLAGIIGGS  
30           151   TNAMSPILLI FLLSETENKN RIVKSSNLGY LLAKIVQIYM LRDQYWLLNK  
         201   SEYGLIFLLS VLSVIGLYVG IRLRTKISPN FFKMLIFIVL LVLALKIGHS  
         251   GLIKL\*

Computer analysis of this amino acid sequence reveals three putative transmembrane domains. A corresponding ORF from strain A of *N.meningitidis* was also identified:

#### Homology with a predicted ORF from *N.meningitidis* (strain A)

35   ORF51 shows 96.7% identity over a 150aa overlap with an ORF (ORF51a) from strain A of *N.meningitidis*:

					10	20	30
orf51.pep					MAIITLYYSV	NGILNVCACA	KNIQVVANNK
40	orf51a	YKLLAIGSVVGSILGVKLL	LLILPVSWLLLL	MAIITLYYSV	NGILNVCACA	KNIQVVANNK	
		80	90	100	110	120	130
			40	50	60	70	80
orf51.pep			NMVLFGLXX	IIGGSTNAMSPILLI	FLLSETENKNRIVKSSNLCYLLAKIVQIYMLRDQY		
45	orf51a		NMVLFGLAGI	IIGGSTNAMSPILLI	FLLSETENKNRIAKSSNLCYLLAKIVQIYMLRDQY		
		140	150	160	170	180	190
			100	110	120	130	140
50	orf51.pep		WLLNKSEYXL	IFLLSVLSVIGLYVGIRLRTKISPNEFKMLIFIVLLVLALKIGHSGLIKL			
	orf51a		WLLNKSEYGLI	FLLSVLSVIGLYVGIRLRTKISPNEFKMLIFIVLLVLALKIGYSGLIKL			
		200	210	220	230	240	250

ORF51-1 and ORF51a show 99.2% identity in 255 aa overlap:

```

5      orf51a.pep  MQEIMQSIVFVAAAILHGITGMGFMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN
      orf51-1     MQEIMQSIVFVAAAILHGITGMGFMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN

      orf51a.pep  NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLILPVSWLLLLMAIITLYYSVNGILN
      orf51-1     NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLILPVSWLLLLMAIITLYYSVNGILN

10     orf51a.pep  VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIAKSSNLCY
      orf51-1     VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIVKSSNLCY

      orf51a.pep  LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNEFKMLIFIVL
15     orf51-1     LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNEFKMLIFIVL

      orf51a.pep  LVLALKIGYSGLIKLX
20     orf51-1     LVLALKIGHSGLIK LX

```

The complete length ORF51a nucleotide sequence <SEQ ID 77> is:

```

1      ATGCAAGAAA TAATGCAATC TATCGTTTTT GTTGCTGCCG CAATACTGCA
51     CGGAATTACA GGCATGGGAT TTCCGATGCT CGGTACAACC GCATTGGCTT
25     101 TTATCATGCC ATTGCTAAG GTTGTTCCT TGGTGGCATT ACCAAGCCTG
      151 TTAATGAGCT TGTGTGTTCT ATGCAGCAAT AACAAAAAGG GTTTTGGCA
      201 AGAGATTGTT TATTATTTAA AAACCTATAA ATTGCTTGCT ATCGGCAGCG
      251 TCGTTGGCAG CATTTTGGGG GTGAAGTTGC TTTTGATACT TCCAGTGTCT
      301 TGGCTGCTTT TACTGATGGC AATCATTACA TTGTATTATT CTGTCAATGG
30     351 TATTTTAAAT GTATGTGCAA AAGCAAAAAA TATTCAAGTA GTTGCCAATA
      401 ATAAGAATAT GGTCTTTTTT GGGTTTTTGG CAGGCATCAT CGGCGGTCA
      451 ACCAATGCCA TGTCTCCCAT ATTGTTAATA TTTTGGCTTA GCGAAACAGA
      501 GAATAAAAAAT CGTATCGCAA AATCAAGCAA TCTATGCTAT CTTTGGCAA
      551 AAATTGTTCA AATATATATG CTAAGAGACC AGTATTGGTT ATTAAATAAG
35     601 AGTGAATACG GTTTAATATT TTTACTGTCC GTATTGTCTG TTATTGGATT
      651 GTATGTTGGA ATTCGGTTAA GGAATAAGAT TAGCCCAAAT TTTTAAAAA
      701 TGTTAATTTT TATTGTTTAA TTGGTATTGG CTCTGAAAAT CGGGTATTCA
      751 GGTTTAATCA AACTTTAA

```

This encodes a protein having amino acid sequence <SEQ ID 78>:

```

40     1      MQEIMQSIVF VAAAILHGIT GMGFMLGTT ALAFIMPLSK VVALVALPSL
      51     LMSLLVLCSN NKKGFWQEIV YYLKTYKLLA IGSVVGSILG VKLLILPV
      101    WLLLLMAIIT LYYSVNGILN VCAKAKNIQV VANNKNMVLFG FLAGIIGGS
      151    TNAMSPILLI FLLSETENKN RIAKSSNLCY LLAKIVQIYM LRDQYWLLNK
      201    SEYGLIFLLS VLSVIGLYVG IRLRTKISPN FFKMLIFIVL LVLALKIGYS
      251    GLIKL*

```

45 Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

### Example 18

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 79>

```

50     1      ATGAGACATA TGAAAATACA AAATTATTTA CTAGTATTTA TAGTTTTACA
      51     TATAGCCTTG ATAGTAATTA ATATAGTGTT TGGTATTTTT GTTTTCTAT
      101    TTGATTTTTT TGCCTTTTTG TTTTGTGCAA ACGTCTTTCT TGCTGTAAAT
      151    TTATTATTTT TAGAAAAAAC CATAAAAAAC AAATTATTGT TTTTATTGCC
      201    GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA
      251    AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT
55     301    ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA

```



```

351 TGGATATGCT AAATTAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG
401 AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA
451 AGATTAAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT
501 TATAAAATTT GTCAGG..

```

5 This corresponds to the amino acid sequence <SEQ ID 80; ORF82>:

```

1 MRHMKIQNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN
51 LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI
101 TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI
151 RLSLVCGIHS YAPCANFIKF VR..

```

10 Further work revealed the complete nucleotide sequence <SEQ ID 81>:

```

1 ATGAGACATA TGAAAAATAA AAATTATTTA CTAGTATTTA TAGTTTTACA
51 TATAGCCTTG ATAGTAATTA ATATAGTGTT TGGTTATTTT GTTTTCTAT
101 TTGATTTTTT TCGGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT
151 TTATTATTTT TAGAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC
15 201 GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA
251 AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT
301 ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA
351 TGGATATGCT AAATTAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG
401 AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA
20 451 AGATTAAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT
501 TATAAAATTT GCAAAAAAAC CTGTTAAAT TTATTTTAT AATCAACCTC
551 AAGGAGATTT TATAGATAAT GTAATATTG AAATTAATGA TGGAAACAAA
601 AGTTTGTACT TGTTAGATAA GTATAAACA TTTTCTTTA TTGAAAACAG
25 651 TGTTTGTATC GTATTAATTA TTTTATATTT AAAATTTAAT TTGCTTTTAT
701 ATAGGACTTA CTTCAATGAG TTGGAATAG

```

This corresponds to the amino acid sequence <SEQ ID 82; ORF82-1>:

```

1 MRHMKNKNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN
51 LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI
30 101 TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI
151 RLSLVCGIHS YAPCANFIKF AKKPVKIYFY NQPQGD FIDN VIFEINDGNK
201 SLYLLDKYKT FFLIENSVCI VLIILYLKFN LLLYRTYFNE LE*

```

Computer analysis of this amino acid sequence reveals a predicted leader peptide.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

Homology with a predicted ORF from *N.meningitidis* (strain A)

35 ORF82 shows 97.1% identity over a 172aa overlap with an ORF (ORF82a) from strain A of *N.meningitidis*:

```

10      20      30      40      50      60
orf82.pep MRHMKIQNYLLVFIVLHIALIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
40 orf82a MRHMKNKNYLLVFIVLHITLIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
10      20      30      40      50      60
70      80      90      100     110     120
orf82.pep KLLFLLPISIIIIWMVIHISM INIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
45 orf82a KLLFLLPISIIIIWMVIHISM INIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
70      80      90      100     110     120
130     140     150     160     170
orf82.pep KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFVR
50 orf82a KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
130     140     150     160     170     180

```

ORF82a and ORF82-1 show 99.2% identity in 242 aa overlap:

```

5  orf82a.pep  MRHMKNKNYLLVFIVLHITLIVINIVFGYFVFLDFFAFLFFANVFLAVNLLFLEKNIKN
    orf82-1    MRHMKNKNYLLVFIVLHIALIVINIVFGYFVFLDFFAFLFFANVFLAVNLLFLEKNIKN

10 orf82a.pep  KLLFLLPISIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
    orf82-1    KLLFLLPISIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA

15 orf82a.pep  KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
    orf82-1    KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY

20 orf82a.pep  NQPQGDFIDNVIFEINDGKKSPLYLLDKYKTFFLIENSVCIVLIIILYLKFNLLLYRTYFNE
    orf82-1    NQPQGDFIDNVIFEINDGNKSPLYLLDKYKTFFLIENSVCIVLIIILYLKFNLLLYRTYFNE

    orf82a.pep  LEX
    orf82-1    LEX

```

The complete length ORF82a nucleotide sequence <SEQ ID 83> is:

```

25 1  ATGAGACATA  TGAAAAATAA  AAATTATTTA  CTAGTATTTA  TAGTTTACAT
    51  TATAACCTTG  ATAGTAATTA  ATATAGTGTT  TGGTTATTTT  GTTTTCTAT
    101  TTGATTTTTT  TCGCTTTTGG  TTTTTCGCAA  ACGTCTTTCT  TGCTGTAAAT
    151  TTATTATTTT  TAGAAAAAAA  CATAAAAAAC  AAATTATTGT  TTTTATTGCC
    201  GATTTCTATT  ATTATATGGA  TGGTAATTCA  TATTAGTATG  ATAAATATAA
    251  AATTTTATAA  ATTTGAGCAT  CAAATAAAGG  AACAAAATAT  ATCCTCGATT
    301  ACTGGGGTGA  TAAAACCACA  TGATAGTTAT  AATTATGTTT  ATGACTCAAA
    351  TGGATATGCT  AAATTAAAAA  ATAATCATAG  ATATGGTAGG  GTAATTAGAG
    401  AAACACCTTA  TATTGATGTA  GTTGCATCTG  ATGTTAAAAA  TAAATCCATA
    451  AGATTAAGCT  TGGTTTGTGG  TATTCATTCA  TATGCTCCAT  GTGCCAATTT
    501  TATAAAATTT  GCAAAAAAAC  CTGTTAAAAA  TTATTTTAT  AATCAACCTC
    551  AAGGAGATTT  TATAGATAAT  GTAATATTG  AAATTAATGA  TGGAAAAAAA
35  601  AGTTTGTACT  TGTTAGATAA  GTATAAAACA  TTTTTCCTTA  TTGAAAACAG
    651  TGTTGTATC  GTATTAATTA  TTTTATATTT  AAAATTTAAT  TTGCTTTTAT
    701  ATAGGACTTA  CTTCAATGAG  TTGGAATAG

```

This encodes a protein having amino acid sequence <SEQ ID 84>:

```

40 1  MRHMKNKNYL  LVFIVLHITL  IVINIVFGYF  VFLDFFAFL  FFANVFLAVN
    51  LLFLEKNIKN  KLLFLLPISI  IIWMVIHISM  INIKFYKFEH  QIKEQNISSI
    101  TGVIKPHDSY  NYVYDSNGYA  KLKDNHRYGR  VIRETPYIDV  VASDVKNKSI
    151  RLSLVCGIHS  YAPCANFIKF  AKKPVKIYFY  NQPQGDFIDN  VIFEINDGKK
    201  SLYLLDKYKT  FFLIENSVCI  VLIILYLKFN  LLLYRTYFNE  LE*

```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

### Example 19

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 85>

```

50 1  . .ACCCCCAACA  GCGTGACCGT  CTTGCCGTCT  TTCGGCGGAT  TCGGGCGTAC
    51  CGGCGCGACC  ATCAATGCAG  CAGGCGGGGT  CGGCATGACT  GCCTTTTCGA
    101  CAACCTTAAT  TTCCGTAGCC  GAGGCGCGGG  TTGTAGAGCT  GCAGGCCGTG
    151  AGAGCCAAAG  CCGTCAATGC  AACC GCCGCT  TGCATTTTTA  CGGTCTTGAG
    201  TAAGGACATT  TTCGATTTCC  TTTTATTTT  CCGTTTTCAG  ACGGCTGACT
    251  TCCGCCTGTA  TTTTCGCCAA  AGCCATGCCG  ACAGCGTGCG  CCTTGACTTC
    301  ATATTTAAAA  GCTTCCGCGC  GTGCCAGTTC  CAGTTCGCGC  GCATAGTTT
55  351  GAGCCGACAA  CAGCAGGGCT  TCGCCCTTGT  CGCGCTCCAT  CTTGTCGATG

```

```

401   ACCGCCTGCA GCTTCGCAAA TGCCGACTTG TAGCCTTGAT GGTGCGACAC
451   AGCCAAGCCC GTGCCGACAA GCGCGATAAT GGCAATCGGT TGCCAGTAAT
501   TCGCCAGCAG TTTCACGAGA TTCATTCTCG ACCTCCTGAC GCTTCACGCT
551   GA

```

5 This corresponds to the amino acid sequence <SEQ ID 86; ORF124>:

```

1   ..TPNSVTVLPS FGGFGRGTGAT INAAGGVGMT AFSTTLISVA EGAVVELQAV
51  RAKAVNATAA CIFTVLISKDI FDFLFIFRFQ TADFRLYFRQ SHADSVRLDF
101 IFKSFRACQF QFARIVLSRQ QQGLRLVALH LVDDRLLQLRK CRLVALMVRH
151 SQARADKRDN GNRLPVIRQQ FHEIHSRPPD ASR*

```

10 Computer analysis of this amino acid sequence predicts a transmembrane domain.

Further work revealed the complete nucleotide sequence <SEQ ID 87>:

```

1   ATGACTGCCT TTTCGACAAC CTTAATTTCC GTAGCCGAGG GCGCGGTTGT
51  AGAGCTGCAG GCCGTGAGAG CCAAAGCCGT CAATGCAACC GCCGCTTGCA
101 TTTTACCGT CTTGAGTAAG GACATTTTCG ATTTCCTTTT TATTTTCCGT
151 TTTCAGACGG CTGACTTCCG CCTGTTTTTT CGCCAAAGCC ATGCCGACAG
201 CGTGCGCCTT GACTTCATAT TTTTATAGCTT CCGCGCGTGC CAGTTCAGT
251 TCGCGCGCAT AGTTTTGAGC CGACAACAGC AGGGCTTGCG CCTTGTCGCG
301 CTCCATCTTG TCGATGACCG CCTGCTGCTT CGCAAATGCC GACTTGTAGC
351 CTTGATGGTG CGACACAGCC AAGCCCCTGC CGACAAGCGC GATAATGGCA
401 ATCGGTTGCC AGTTATTCGC CAGCAGTTTC ACGAGATTCA TTCTCGACCT
451 CCTGACGCTT CACGCTGA

```

This corresponds to the amino acid sequence <SEQ ID 88; ORF124-1>:

```

1   MTAFTTLIS VAEGAVVELQ AVRAKAVNAT AACIFTVLISK DIFDFLFIFR
51  FQTADFRLEF RQSHADSVRL DFIFFSFRAC QFQFARIVLS RQQQGLRLVA
101 LHLVDDRLLL RKRLVALMV RHSQARADKR DNGNRLPVIR QFHEIHSRP
151 PDASR*

```

A corresponding ORF from strain A of *N.meningitidis* was also identified:

#### Homology with a predicted ORF from *N.meningitidis* (strain A)

ORF124 shows 87.5% identity over a 152aa overlap with an ORF (ORF124a) from strain A of *N.meningitidis*:

```

30
      10      20      30      40      50      60
orf124.pep  TPNSVTVLPSFGGFGRGTGATINAAGGVGMTAFSTTLISVAEGAVVELQAVRAKAVNATAA
orf124a      MTAFTTLISVAEGALVELQAVMAKAVNTTAA
35
      70      80      90      100     110     120
orf124.pep  CIFTVLISKDIFDFLFIFRFQTADFRLYFRQSHADSVRLDFIFKSFRACQFQFARIVLSRQ
orf124a      CIFTVLISKDIFDFLFIFRFQTADFRLYFRQSHADGVRLDFIFFSFRTRLFQFAGVLSRQ
40
      40      50      60      70      80      90
orf124.pep  QQGLRLVALHFLVDDRLLQLRKRLVALMVRHSQARADKRDNGNRLPVIRQQFHEIHSRPPD
orf124a      QQGLRLVALHFLNDRLLLRKSRLVALMVRHRQTRADKRDDGNRLPVIRQQFHEIHSRPPD
45
      100     110     120     130     140     150
orf124.pep  ASRX
orf124a      VX
50

```

ORF124a and ORF124-1 show 89.5% identity in 152 aa overlap:

```

    orf124-1.pep    MTAFSTTLISVAEGAVVELQAVRAKAVNATAACIFTVLSDKIDFDLFIFRFQTADFRLEFF
    orf124a         MTAFSTTLISVAEGALVELQAVMAKAVNTTAACIFTVLSDKIDFDLFIFRFQTADFRLEFF
5   orf124-1.pep    RQSHADSVRLDFFFSFRACQFQFARIVLSRQQQGLRLVALHLVDDRLLLRKRLVALMV
    orf124a         RQSHADGVRLDFFFSFRTRLFQFAGVVLRSRQQQGLRLVALHFLNDRLLLRKSRLVALMV
10  orf124-1.pep    RHSQARADKRDNGNRLPVIRQQFHEIHSRPPDASRX
    orf124a         RHRQTRADKRDDGNRLPVIRQQFHEIHSRPPDVX

```

The complete length ORF124a nucleotide sequence <SEQ ID 89> is:

```

1   ATGACCGCCT TTTGACAAC CTTAATTTCC GTAGCCGAGG GCGCGCTTGT
51  AGAGCTGCAA GCCGTGATGG CCAAAGCCGT CAATACAACC GCCGCCTGCA
15  TTTTACGGT CTGAGTAAG GACATTTTCG ATTTCTTTT TATTTTCCGT
151 TTTTACGGT CTGACTTCCG CCTGTTTTT CGCCAAAGCC ATGCCGACGG
201 CGTGCGCCTT GACTTCATAT TTTTGTAGCT CCGCACGCGC CTGTTCCAGT
251 TCGCGGGCGT AGTTTGTAGC CGACAACAGC AGGGCTTGCG CCTGTGTCGG
20  CTTCAATTTT TCAATGACCG CCTGCTGCTT CGCAAAGCC GACTTGTAGC
351 CTTGATGGTG CGACACCGCC AAACCCGTGC CGACAAGCGC GATGATGGCA
401 ATCGGTTGCC AGTTATTCGC CAGCAGTTTC ACGAGATTCA TTCTCGACCT
451 CCTGACGTTT GA

```

This encodes a protein having amino acid sequence <SEQ ID 90>:

```

1   MTAFSTTLIS VAEGALVELQ AVMAKAVNTT AACIFTVLSK DIFDRLFIFR
25  51  FQTADFRLEFF RQSHADGVRL DFIFFSERTR LFQFAGVVLS RQQQGLRLVA
    101 LHFLNDRLLL RKSRLVALMV RHRQTRADKR DDGNRLPVIR QQFHEIHSRP
    151 PDV*

```

ORF124-1 was amplified as described above. Figure 7 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF124-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

It will be appreciated that the invention has been described by means of example only, and that modifications may be made whilst remaining within the spirit and scope of the invention.

TABLE I – PCR primers

ORF	Primer	Sequence	Restriction sites
<b>ORF 38</b>	Forward Reverse	CGCGGATCCCATATG-TCGCCGCAAAATTCCGA CCCGCTCGAG-TTTTGCCGCGTTAAAAGC	BamHI-NdeI XhoI
<b>ORF 40</b>	Forward Reverse	CGCGGATCCCATATG-ACCGTGAAGACCGCC CCCGCTCGAG-CCACTGATAACCGACAGA	BamHI-NdeI XhoI
<b>ORF 41</b>	Forward Reverse	CGCGGATCCCATATG-TATTTGAAACAGCTCCAAG CCCGCTCGAG-TTCTGGGTGAATGTTA	BamHI-NdeI XhoI
<b>ORF 44</b>	Forward Reverse	GCGGATCCCATATG-GGCACGGACAACCCC CCCGCTCGAG-ACGTGGGGAACAGTCT	BamHI-NdeI XhoI
<b>ORF 51</b>	Forward Reverse	GCGGATCCCATATG-AAAAATATTCAAGTAGTTGC CCCGCTCGAG-AAGTTTGATTAAACCCG	BamHI-NdeI XhoI
<b>ORF 52</b>	Forward Reverse	CGCGGATCCCATATG-TGCCAACCGCAATCCG CCCGCTCGAG-TTTTTCAGCTCCGGCA	BamHI-NdeI XhoI
<b>ORF 56</b>	Forward Reverse	GCGGATCCCATATG-GTTATCGGAATATTACTCG CCCGCTCGAG-GGCTGCAGAAGCTGG	BamHI-NdeI XhoI
<b>ORF 69</b>	Forward Reverse	CGCGGATCCCATATG-CGGACGTGGTTGGTTTT CCCGCTCGAG-ATATCTTCCGTTTTTTTCAC	BamHI-NdeI XhoI
<b>ORF 82</b>	Forward Reverse	CGCGGATCCGCTAGC-GTAAATTTATTATTTTAGAA CCCGCTCGAG-TTCCAATCATTGAAGTA	BamHI-NheI XhoI
<b>ORF 114</b>	Forward Reverse	CGCGGATCCCATATG-AATAAAGGTTTACATCGCAT CCCGCTCGAG-AATCGCTGCACCGGCT	BamHI-NheI XhoI
<b>ORF 124</b>	Forward Reverse	CGCGGATCCCATATG-ACTGCCTTTTCGACA CCCGCTCGAG-GCGTGAAGCGTCAGGA	BamHI-NheI XhoI

**TABLE II – Cloning, expression and purification**

<b>ORF</b>	<b>PCR/cloning</b>	<b>His-fusion expression</b>	<b>GST-fusion expression</b>	<b>Purification</b>
<b>orf 38</b>	+	+	+	<b>His-fusion</b>
<b>orf 40</b>	+	+	+	<b>His-fusion</b>
<b>orf 41</b>	+	n.d.	n.d.	
<b>orf 44</b>	+	+	+	<b>His-fusion</b>
<b>orf 51</b>	+	n.d.	n.d.	
<b>orf 52</b>	+	n.d.	+	<b>GST-fusion</b>
<b>orf 56</b>	+	n.d.	n.d.	
<b>orf 69</b>	+	n.d.	n.d.	
<b>orf 82</b>	+	n.d.	n.d.	
<b>orf 114</b>	+	n.d.	+	<b>GST-fusion</b>
<b>orf 124</b>	+	n.d.	n.d.	

**CLAIMS**

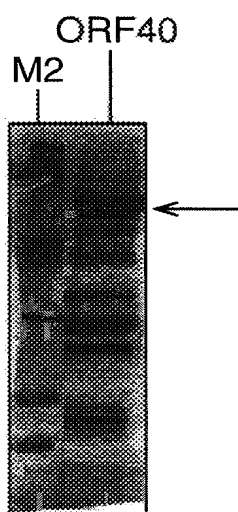
1. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, and 6.
2. A nucleic acid molecule which encodes a protein according to claim 1.
- 5 3. A nucleic acid molecule according to claim 2, comprising a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, and 5.
4. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90.
- 10 5. A protein having 50% or greater sequence identity to a protein according to claim 4.
6. A protein comprising a fragment of an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90.
7. An antibody which binds to a protein according to any one of claims 4 to 6.
- 15 8. A nucleic acid molecule which encodes a protein according to any one of claims 4 to 6.
9. A nucleic acid molecule according to claim 8, comprising a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, and 89.
- 20 10. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, and 89.
11. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to any one of claims 8 to 10.

12. A nucleic acid molecule comprising a nucleotide sequences having 50% or greater sequence identity to a nucleic acid molecule according to any one of claims 8 to 11.
13. A nucleic acid molecule which can hybridise to a nucleic acid molecule according to any one of claims 8 to 12 under high stringency conditions.
- 5 14. A composition comprising a protein, a nucleic acid molecule, or an antibody according to any preceding claim.
15. A composition according to claim 14 being a vaccine composition or a diagnostic composition.
16. A composition according to claim 14 or claim 15 for use as a pharmaceutical.
- 10 17. The use of a composition according to claim 14 in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, particularly *Neisseria meningitidis*.

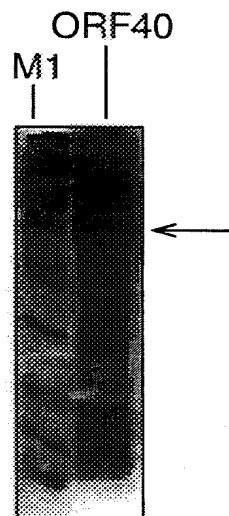


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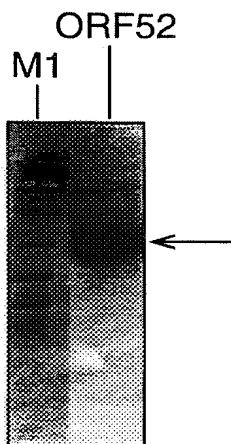
**FIG. 1A**



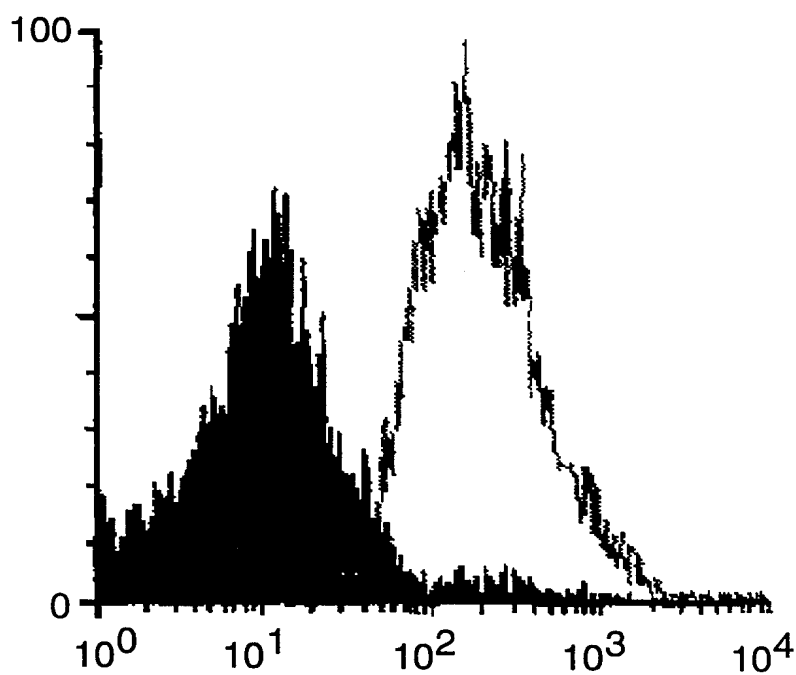
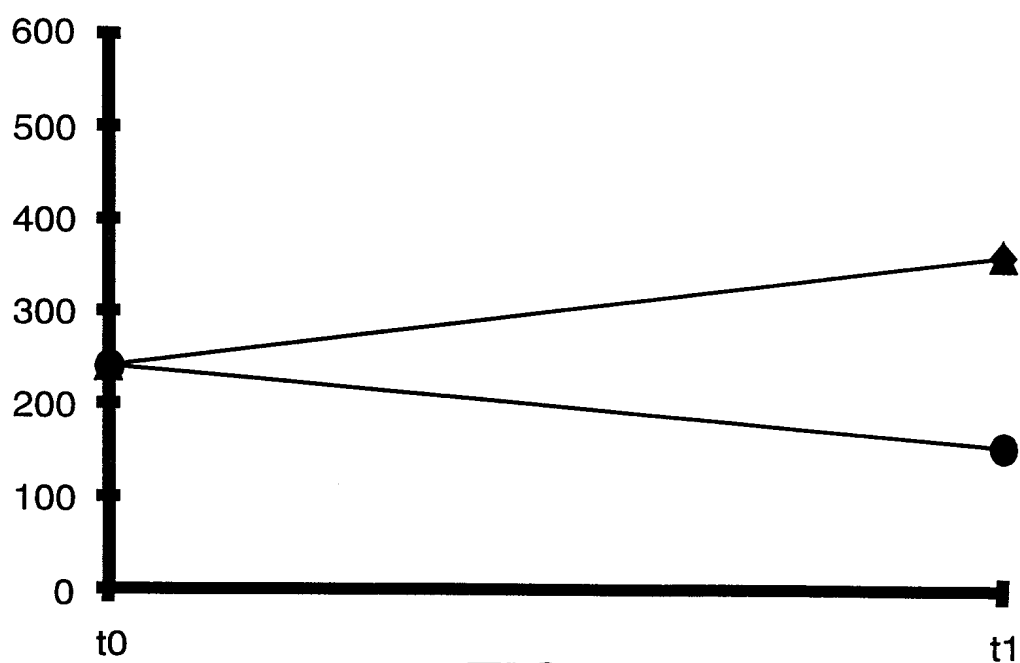
**FIG. 1B**



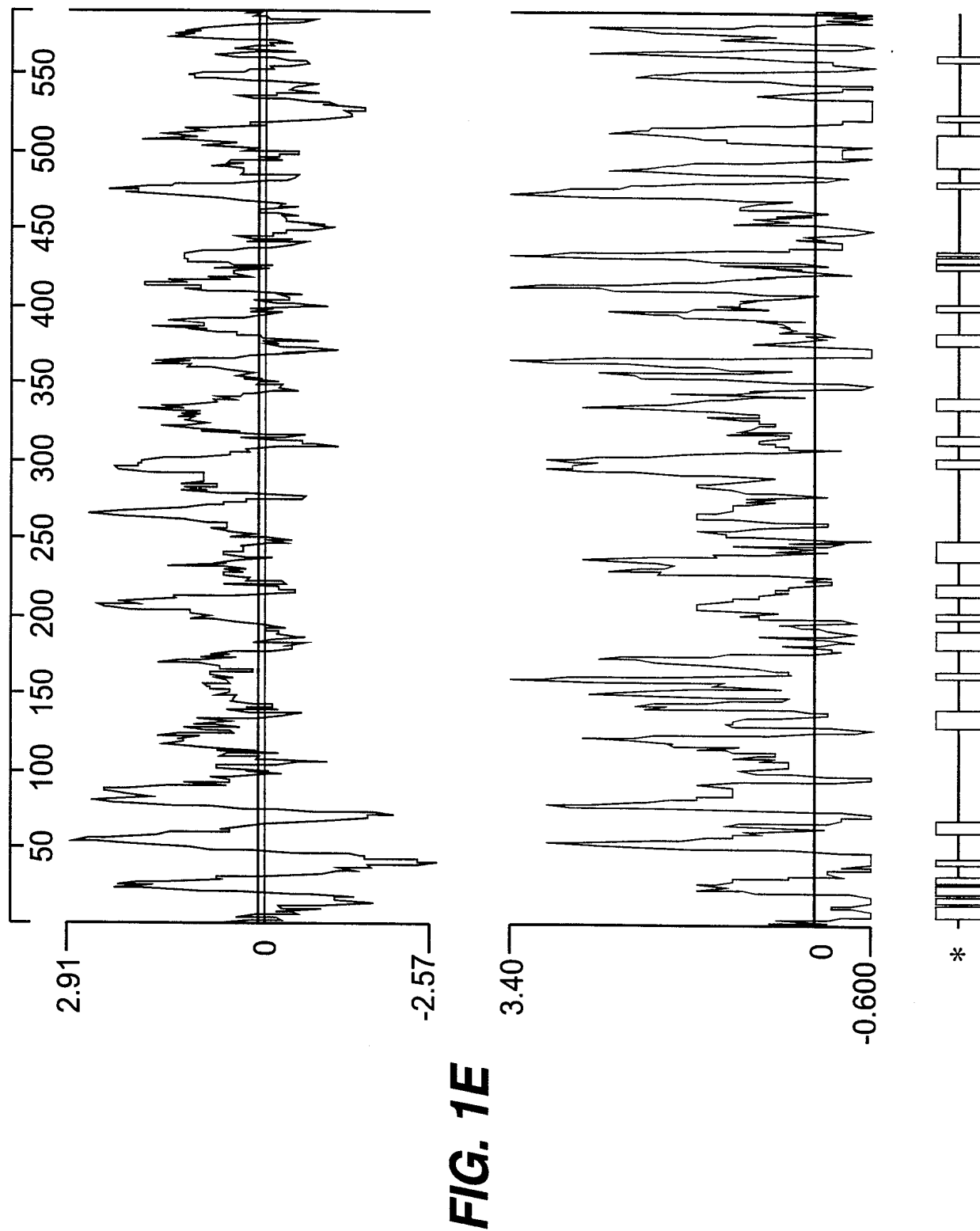
**FIG. 4A**



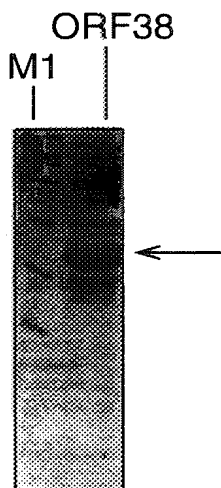
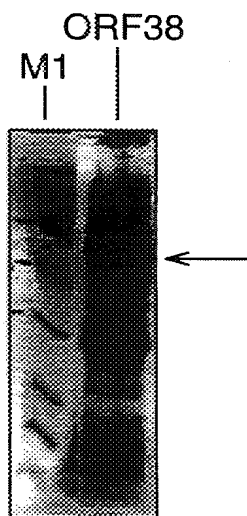
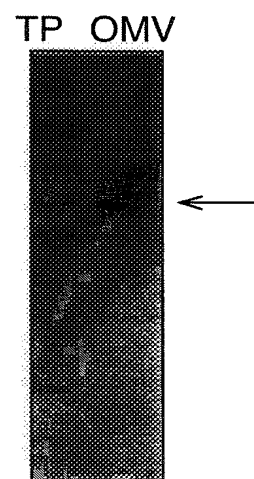
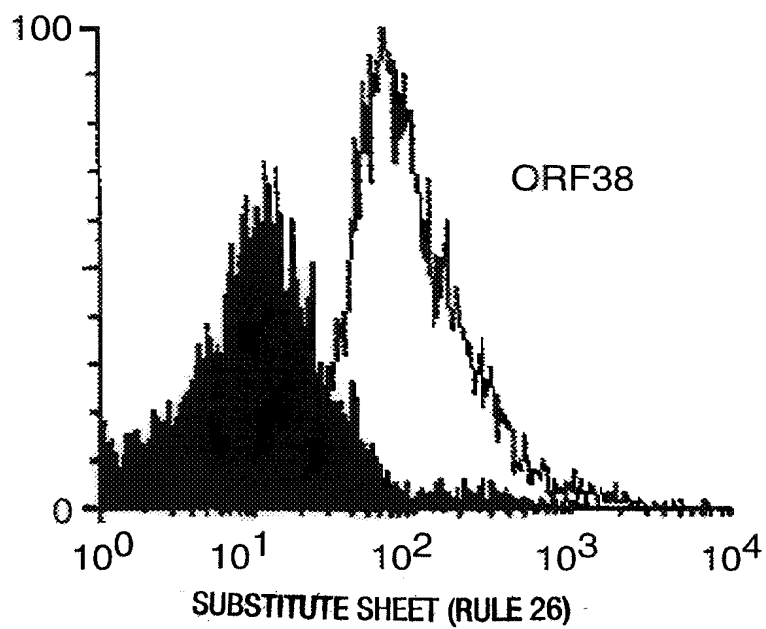
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**FIG. 1C****FIG. 1D**

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**FIG. 2A****FIG. 2B****FIG. 2C****FIG. 2D**

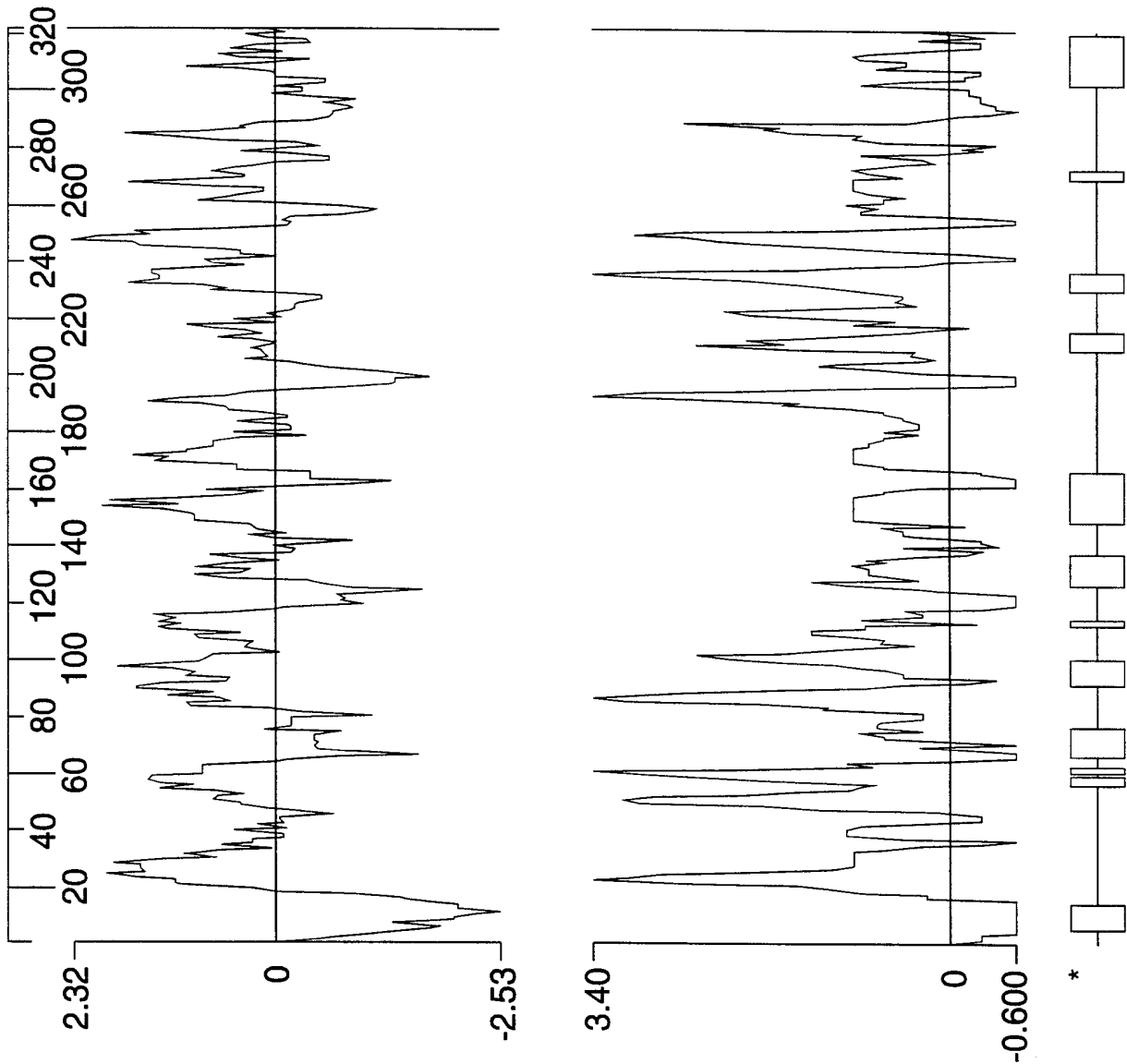
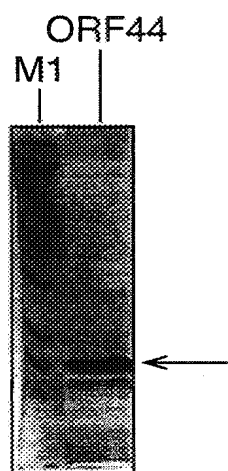


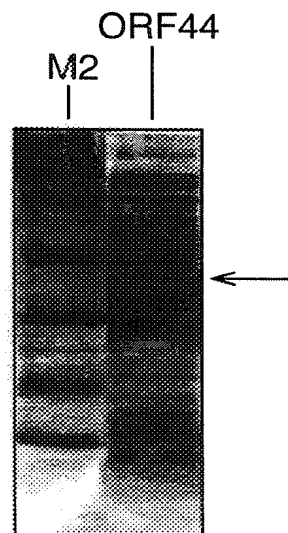
FIG. 2E

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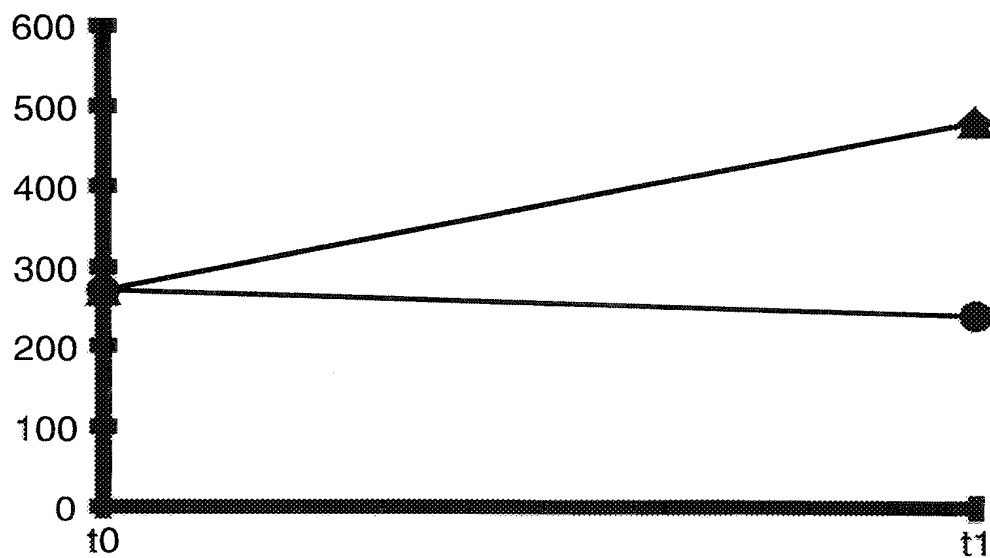
**FIG. 3A**



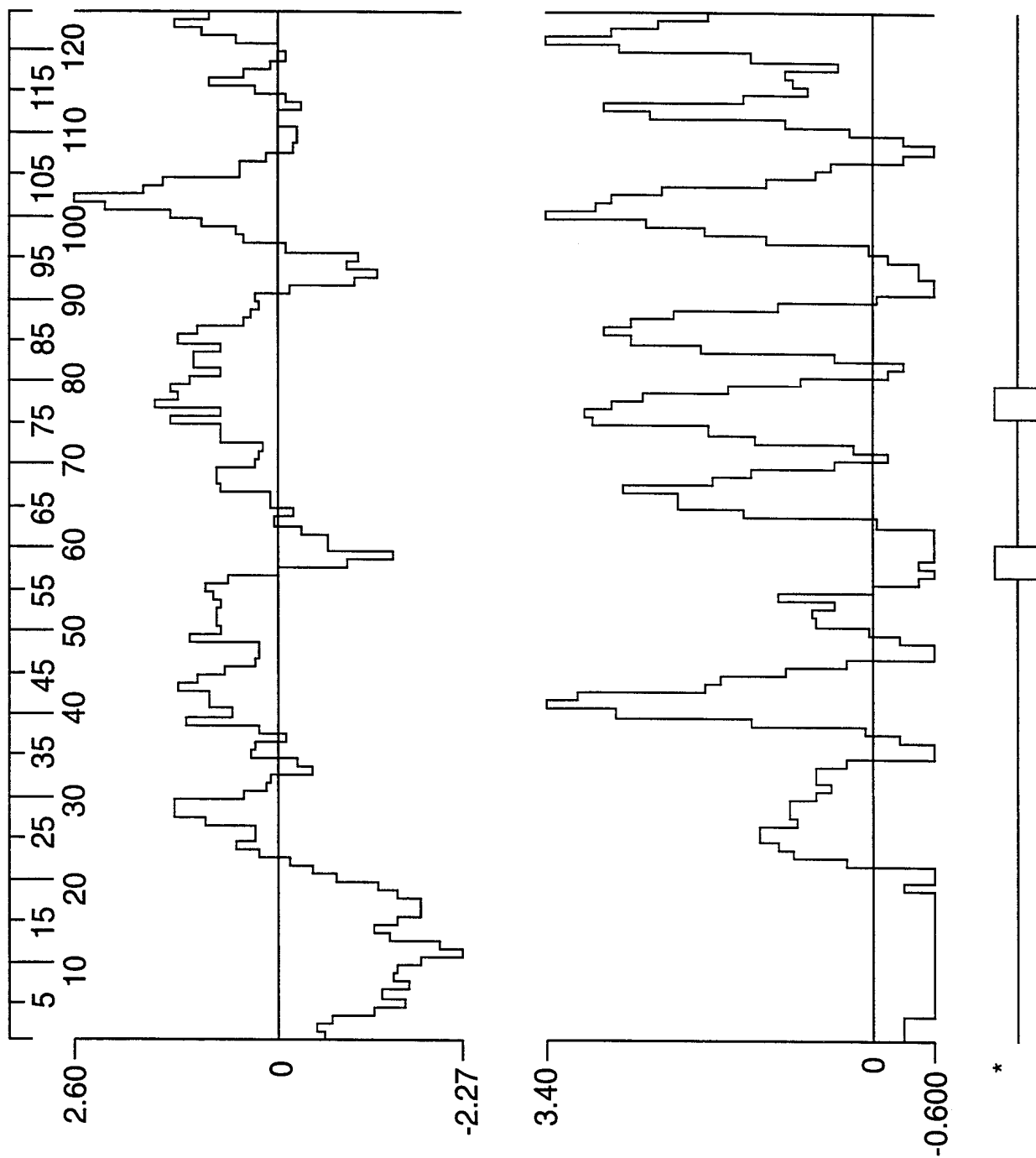
**FIG. 3B**



**FIG. 3C**



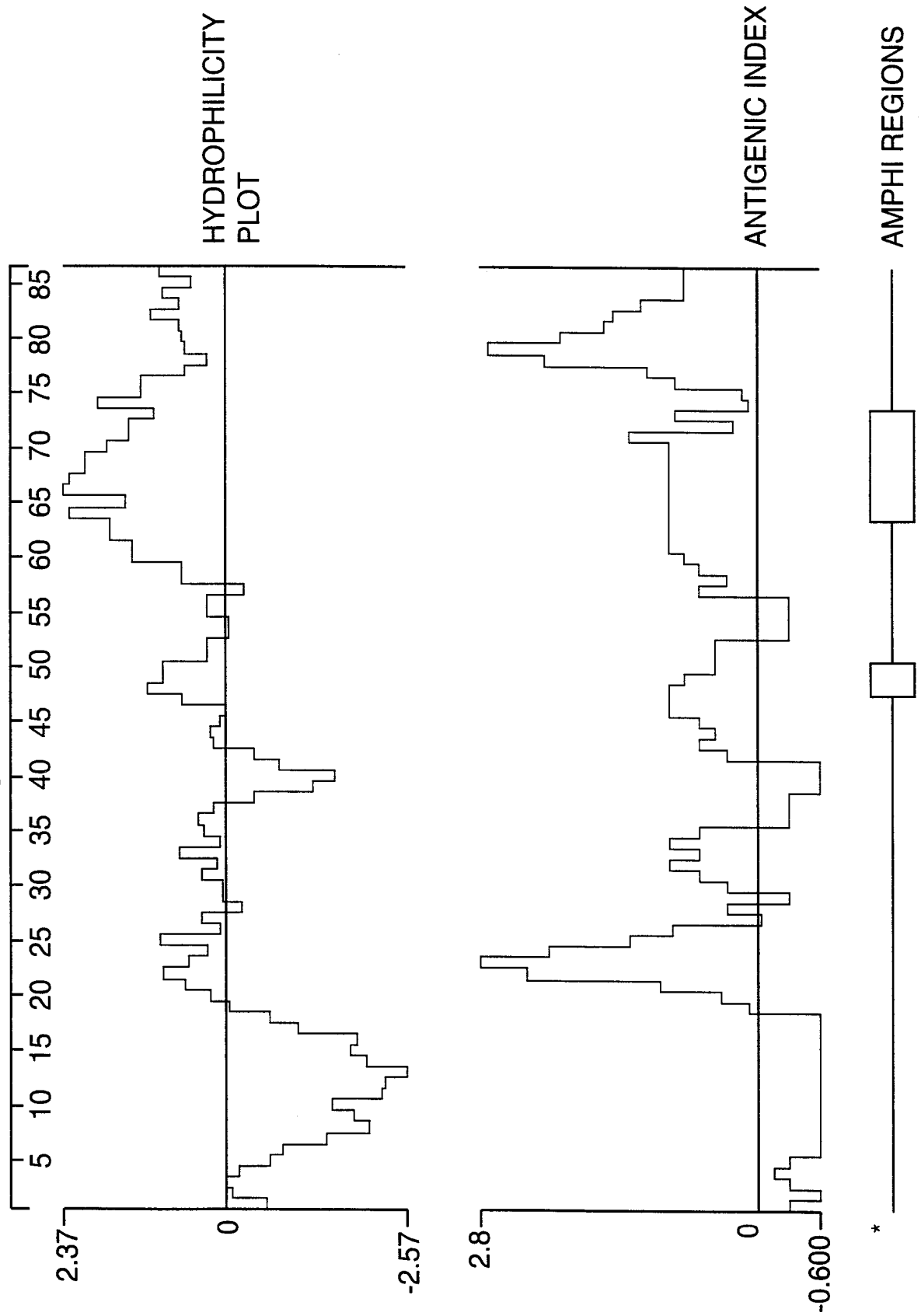
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**FIG. 3D**

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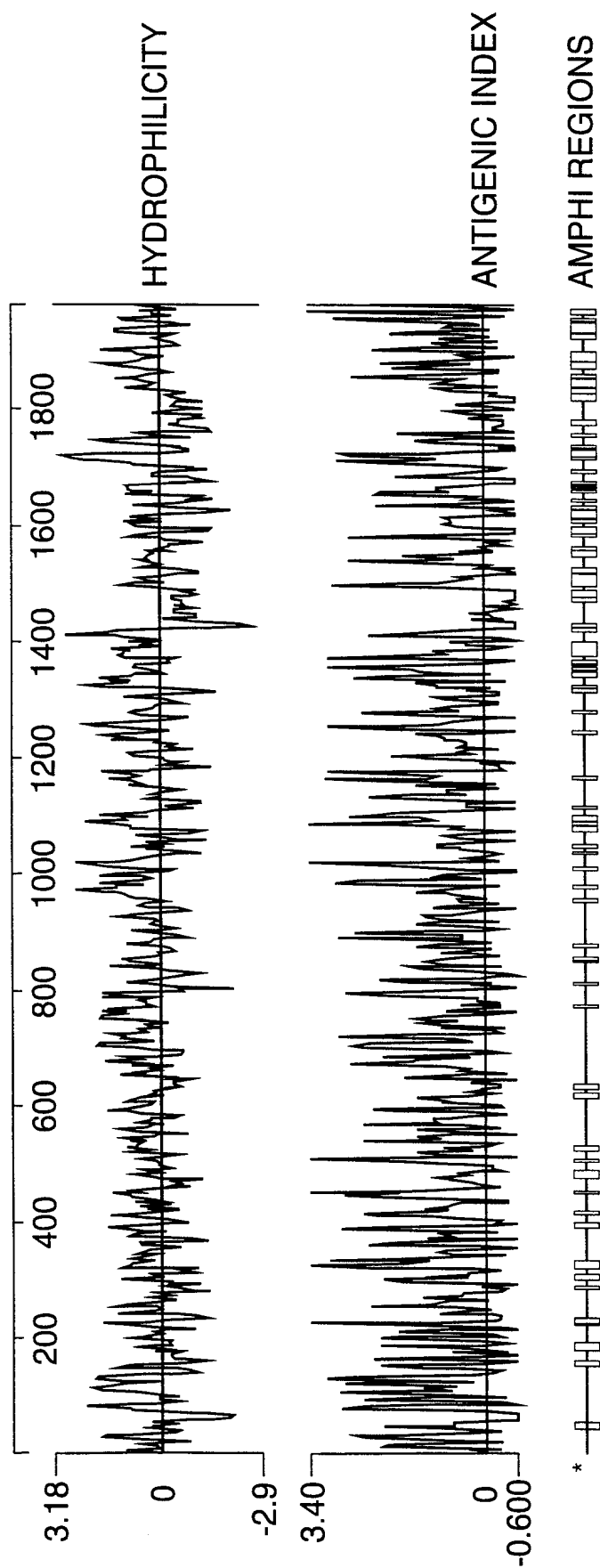
**FIG. 4B**





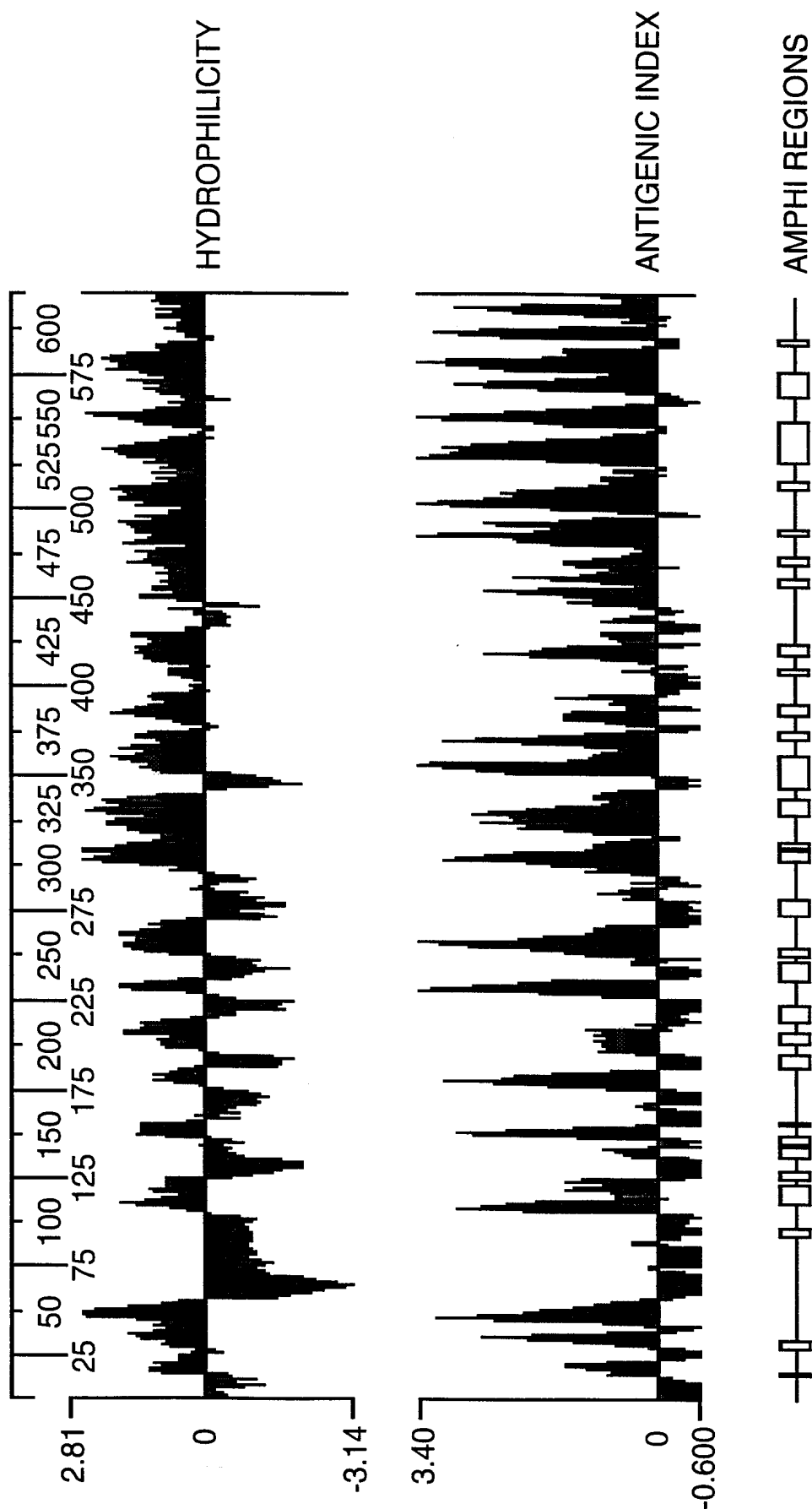
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**FIG. 5**



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**FIG. 6**



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**FIG. 7**

